EFFECTS OF FINGERROOT (*BOESENBERGIA ROTUNDA*) POWDER ON EGG PERFORMANCE, AND QUALITY, EGG YOLK MALONDIALDEHYDE IN LAYING HENS

Mr. Miftah Nurfarid

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Applied Animal Science
Department of Animal Husbandry
FACULTY OF VETERINARY SCIENCE
Chulalongkorn University
Academic Year 2022

ผลของผงกระชายต่อประสิทธิภาพในการวางไข่ คุณภาพไข่ และระดับมาลอนไดแอลดีไฮด์ในไข่แดงของไก่ไข่

นายมิฟทาห์ นูร์ฟาอิด

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสัตวศาสตร์ประยุกต์ ภาควิชาสัตวบาล
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2565

Thesis Title	EFFECTS OF FINGERROOT (*BOESENBERGIA ROTUNDA*) POWDER ON EGG PERFORMANCE, AND QUALITY, EGG YOLK MALONDIALDEHYDE IN LAYING HENS
By	Mr. Miftah Nurfarid
Field of Study	Applied Animal Science
Thesis Advisor	Associate Professor CHACKRIT NUENGJAMNONG, Ph.D.
Thesis Co Advisor	Assistant Professor HATAIRAT PLAIMAST, Ph.D.

Accepted by the FACULTY OF VETERINARY SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the FACULTY OF VETERINARY SCIENCE

(Professor SANIPA SURADHAT, Ph.D.)

THESIS COMMITTEE

..... Chairman

(Assistant Professor ANONGNART ASSAVACHEEP, Ph.D.)

..... Thesis Advisor

(Associate Professor CHACKRIT NUENGJAMNONG, Ph.D.)

..... Thesis Co-Advisor

(Assistant Professor HATAIRAT PLAIMAST, Ph.D.)

..... Examiner

(Associate Professor KRIS ANGKANAPORN, Ph.D.)

..... External Examiner

(Associate Professor Keatisak Soisuwan, Ph.D.)

มิฟทาห์ นูร์ฟาอิด : ผลของผงกระชายต่อประสิทธิภาพในการวางไข่ คุณภาพไข่
และระดับมาลอนไดแอลดีไฮด์ในไข่แดงของไก่ไข่. (EFFECTS OF FINGERROOT (*BOESENBERGIA ROTUNDA*)
POWDER ON EGG PERFORMANCE, AND QUALITY, EGG YOLK MALONDIALDEHYDE IN LAYING HENS)
อ.ที่ปรึกษาหลัก : จักรกริศน์ เนื่องจำนงค์, อ.ที่ปรึกษาร่วม : หทัยรัตน์ พลายมาศ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมผงกระชายในอาหารไก่ไข่ต่อสมรรถนะการให้ผลผลิต คุณภาพไข่ และระดับมาลอนไดอัลดีไฮด์ในไข่แดง โดยทำการวิจัยในไก่ไข่พันธุ์ไฮไลน์บราวน์ที่อายุ 43 สัปดาห์ จำนวน 192 ตัว โดยแบ่งออกเป็น 4 กลุ่ม กลุ่มละ 48 ตัว แต่ละกลุ่มการทดลองมี 8 ซ้ำ ซ้ำละ 6 ตัว กลุ่มการทดลองประกอบด้วย กลุ่มที่ได้รับอาหารพื้นฐาน (กลุ่มควบคุม) กลุ่มที่ได้รับอาหารพื้นฐานเสริมวิตามินอี 250 มิลลิกรัมต่อกิโลกรัม กลุ่มที่ได้รับอาหารพื้นฐานเสริมผงกระชาย 20 กรัมต่อกิโลกรัมอาหาร และกลุ่มที่ได้รับอาหารพื้นฐานเสริมผงกระชาย 40 กรัมต่อกิโลกรัมอาหาร โดยทำการวิเคราะห์สมรรถนะการให้ผลผลิตใน 2 ช่วงการทดลอง ได้แก่ ช่วงอายุ 1 ถึง 3 สัปดาห์ และช่วงอายุ 4 ถึง 6 สัปดาห์ของการเสริมอาหาร ทำการประเมินคุณภาพไข่ 2 ครั้ง ในสัปดาห์ที่ 3 และ 6 หลังการเสริมอาหาร และตรวจวัดระดับมาลอนไดอัลดีไฮด์ในไข่แดง ณ วันที่ 0, 7, 14 และ 21 หลังเก็บรักษาที่อุณหภูมิห้อง ในช่วงแรกของการทดลองพบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติของน้ำหนักไข่ อัตราการวางไข่ มวลไข่ ปริมาณอาหารที่กิน และประสิทธิภาพการเปลี่ยนอาหารเป็นน้ำหนักไข่ ระหว่างกลุ่ม ในสัปดาห์ที่ 4 ถึง 6 ของการทดลองพบว่ากลุ่มที่ได้รับอาหารพื้นฐานเสริมผงกระชาย 20 กรัม มีน้ำหนักไข่มากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ในช่วง 6 สัปดาห์ของการเสริมอาหารพบว่า กลุ่มที่ได้รับอาหารพื้นฐานเสริมผงกระชาย 40 กรัมมีปริมาณอาหารที่กินลดลง แต่มีประสิทธิภาพการเปลี่ยนอาหารเป็นน้ำหนักไข่ดีขึ้น โดยปริมาณการกินอาหารที่ลดลงในกลุ่มการทดลองนี้ไม่ส่งผลกระทบต่ออัตราการวางไข่ น้ำหนักไข่ และมวลไข่นอกจากนี้ยังพบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติของคุณภาพของไข่ในทุกตัวแปร ยกเว้นสีของไข่แดงในสัปดาห์ที่ 3 ของการเสริมอาหาร โดยระดับสีของไข่แดงลดลงอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ในกลุ่มที่ได้รับอาหารพื้นฐานเสริมผงกระชาย 40 กรัม ในการประเมินคุณคุณภาพของไข่ที่ 6 สัปดาห์ พบว่ากลุ่มที่ได้รับอาหารพื้นฐานเสริมผงกระชาย 20 กรัม มีน้ำหนักของไข่ขาวและเปลือกไข่ที่เพิ่มมากขึ้นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) อาหารทดลองไม่ส่งผลต่อระดับมาลอนไดอัลดีไฮด์ในไข่สดหรือไข่ที่เก็บรักษาไว้ อย่างไรก็ตาม ระยะเวลาที่ใช้ในการเก็บรักษาส่งผลกระทบต่อระดับมาลอนไดอัลดีไฮด์ ซึ่งมีระดับสูงขึ้นในวันที่ 21 ของการเก็บรักษา ในงานวิจัยนี้พบว่าวิตามินอีและผงกระชายไม่มีฤทธิ์ต้านอนุมูลอิสระในไข่แดง จากการวิจัยสรุปได้ว่าการเสริมผงกระชายมีประโยชน์ต่อน้ำหนักไข่ ประสิทธิภาพการเปลี่ยนอาหารเป็นน้ำหนักไข่ รวมทั้งน้ำหนักของไข่ขาวและเปลือกไข่

สาขาวิชา	สัตวศาสตร์ประยุกต์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2565	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

6470011631 : MAJOR APPLIED ANIMAL SCIENCE

KEYWORD: Boesenbergia rotunda, egg quality, egg yolk, laying hen, malondialdehyde, performance

Miftah Nurfarid : EFFECTS OF FINGERROOT (*BOESENBERGIA ROTUNDA*) POWDER ON EGG PERFORMANCE, AND QUALITY, EGG YOLK MALONDIALDEHYDE IN LAYING HENS. Advisor: Assoc. Prof. CHACKRIT NUENGJAMNONG, Ph.D. Co-advisor: Asst. Prof. HATAIRAT PLAIMAST, Ph.D.

This study aimed to determine the effect of fingerroot powder supplementation on the performance of laying hens, egg quality, and malondialdehyde in the egg yolk. A total of 192 Hy-Line brown laying hens aged 43 weeks were divided into four treatment groups with 48 hens in each group (8 replicates; 6 birds each). The treatments were the control (basal diet), basal diet supplemented with vitamin E at 250 mg/kg, basal diet plus 20 g fingerroot powder/kg, and basal diet plus 40 g fingerroot powder/kg. Performance parameters were analyzed in the following two experimental period: 1 to 3 week and 4 to 6 week of treatment administration. Egg quality was evaluated twice at 3 and 6 weeks after feeding. The malondialdehyde (MDA) was measured in egg yolk at 0, 7, 14, and 21 days after storage at room temperature. No significant differences were observed in egg weight, laying rate, egg mass, feed intake and feed conversion ratio among the groups during the first experimental period. In the last period 4 to 6 week of experiment, dietary inclusion of 20 g fingerroot powder improved egg weight ($P < 0.05$) compared with control. The performance showed that during 6 weeks of feeding, the group supplemented with 40 g fingerroot powder had a decreased feed intake, but its feed conversion ratio was improved. The low feed intake in this group did not affect the laying rate, egg weight, and egg mass. There was no significant difference in egg quality for all variables except yolk color at 3 weeks of feeding. The yolk color was significantly lower in the feed supplemented with fingerroot powder at 40 g ($P < 0.05$). The egg quality at 6 weeks of feeding showed that 20 g fingerroot powder group had higher albumen and shell weight ($P < 0.05$). The experimental diets did not affect MDA levels in fresh or stored eggs. However, MDA level was affected by storage time and increased at 21 days. In this study, vitamin E or fingerroot powder did not show their antioxidant activity on the egg yolk. In conclusion, the addition of fingerroot powder has beneficial effects on egg weight, FCR, albumen and shell weights.

Field of Study:	Applied Animal Science	Student's Signature .....
Academic Year:	2022	Advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to all those who have supported and contributed to the completion of this thesis. Their assistance and encouragement have been invaluable throughout this journey.

First and foremost, I would like to extend my heartfelt appreciation to my supervisors, Assoc. Prof. Chackrit Nuengjamnong and Assist. Prof. Hatairat Plaimast for their guidance, expertise, and unwavering support. I am grateful for their mentorship and the opportunities they provided for me for doing this research.

I also would like to thank all lecturers whose knowledge and expertise have enriched my academic experience, for teaching me many things, and become the source of my inspiration. I am grateful to all department staff both in the office and farm for their help and kindness. My gratitude also expresses to the laboratory staffs for helping me in doing lab work.

I extend my sincere appreciation to my friends and colleagues for the happiness, togetherness, support, and social life during the study. I would like to express my gratitude to my family for their unconditional love, support, and understanding throughout this journey.

Miftah Nurfarid

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1
INTRODUCTION

Egg is a nutritious food that contains protein, fat, vitamins, and minerals (Li-Chan and Kim, 2008). Egg is a healthy, inexpensive, highly nutritious, and a source of essential polyunsaturated fatty acids (Schreiner et al., 2004). However, the high level of polyunsaturated fatty acids in the egg makes it easier to be oxidized. Polyunsaturated oxidation will be detrimental to the flavor, aroma, nutritional value, and overall quality of the animal product (Khan et al., 2011; Mohamed et al., 2011). One factor that influences consumer preferences is egg quality. Egg quality will decrease depending on the temperature and length of storage (Cimrin et al., 2019). Unsaturated bonds in egg yolk fatty acids react easily with free radicals, resulting in peroxidation products. Both temperature and storage time can influence the rate of peroxidation in eggs (Ramalho and Jorge, 2006).

Antioxidants are substances that can prevent oxidation and decrease free radicals in the body either directly or indirectly (Liu et al., 2014). To stop the oxidation of the fatty acids in egg yolks, antioxidants were added to the feed of laying hens (Ahmad et al., 2015). Antioxidants were reported to positively affect poultry performance (Jang et al., 2008). Zhao et al. (2011) reported that the antioxidant can improve production performance in birds. Therefore, the antioxidant capacity of the egg can be enriched by antioxidant supplementation to reduce lipid peroxidation of the egg yolk (Ngo et al., 2011). Butylated hydroxyanisole and butylated hydroxytoluene, two synthetic antioxidants, have proved to be unfavorable side effects, including liver damage and cancer, especially in long-term usage. The discovery and research on the utilization of natural antioxidants are important (Ser et al., 2016).

The utilization of antioxidants derived from plants has been reported to maintain animal health and protect animal products from peroxidation (Wenk, 2003). Polyphenols are the most common compounds found in plants, with approximately 8000 identified compounds (Tufarelli et al., 2017). Polyphenols can be found in grains, vegetables, fruit, tea, and a variety of plant parts such as flowers, leaves, roots, seeds, and fruits (Petti and Scully, 2009). Plant polyphenols are classified into two types:

flavonoids and non-flavonoids (Surai, 2014). Polyphenols have been shown to act as exogenous antioxidants as the first line of defense for cells against free radicals, thereby protecting against oxidative damage (Procházková et al., 2011; Lipiński et al., 2017). They protect cells from free radicals by inhibiting pro-oxidant enzyme activity, increasing antioxidant enzyme activity, directly scavenging free radicals, and chelating metal ions to limit reactive hydroxyl radicals (HO) formation. Orhan and Olmez (2011) reported that supplementation of a herbal mixture (0.5%) containing thyme in the laying hens' diet reduced malondialdehyde in egg yolk after 56 days of storage. Fascina et al. (2017) also reported that the malondialdehyde levels in the intestine and serum of chickens fed with herbal antioxidants were decreased. Previous studies showed that the dietary addition of plant extracts in laying hens increased the activity of antioxidant enzymes in heat-stress conditions and also increased the oxidative stability of eggs stored at 25 °C (Batista et al., 2017; Torki et al., 2018).

According to the secondary metabolite composition, *B*oesenbergia *rotunda* contains a large number of flavonoids (Chahyadi et al., 2014). This plant is reported its potential as an antioxidant by increasing antioxidant enzyme activity (Fahey and Stephenson, 2002). Methanolic extract and active compounds of *B. rotunda* were able to scavenge free radicals and reduce malondialdehyde (Abdelwahab et al., 2011; Kim et al., 2012). Therefore, *B. rotunda* has a potential as a feed supplement in laying hens' diets to improve the performance, egg quality, and lipid oxidative stability of egg yolks thereby increasing the nutritional quality and shelf life of eggs.

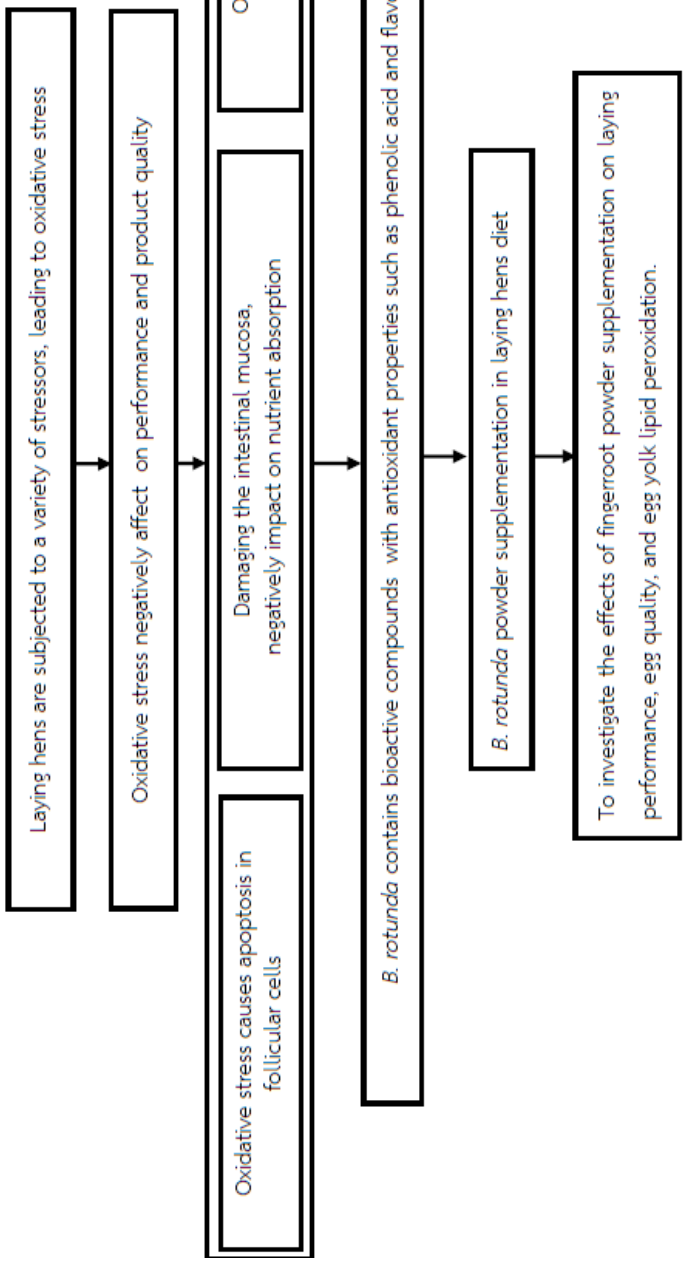
Objectives of study

The study aims to investigate the effect of fingerroot powder supplementation in the diet on laying hen performance, egg quality, and egg yolk malondialdehyde.

Hypothesis

Supplementation of fingerroot powder in the laying hen's diet can improve the laying performance, egg quality, and decrease egg yolk malondialdehyde.

Conceptual framework



Advantages of Study

To reveal the potential of the rhizome powder of *B. rotunda* as a feed supplement to improve the laying performance, egg quality, and reduce egg yolk malondialdehyde. This research is expected to provide additional information regarding the use of natural feed supplements derived from a plant that can increase animal product value and consumer preference.

CHAPTER 2

LITERATURE REVIEW

1. Free radical, oxidative stress, and antioxidant

Free radicals are molecules or compounds that are unstable due to the presence of one or more unpaired electrons. An atom of hydrogen with only one proton and one electron is a simple example of a free radical (Halliwell, 1996). Reactive oxygen species (ROS) are one of the free radicals that can exist independently and contain one or more unpaired electrons. Generally, ROS and reactive nitrogen species (RNS) are the primary free radicals that participate in various metabolic reactions in the body. However, some ROS are produced in the free radical reaction process and do not strictly belong to free radicals, though they can directly or indirectly trigger the free radical reaction. Reactive oxygen species include the superoxide ion, hydrogen peroxide, and hydroxyl radical. The concentration of ROS is a direct marker reflecting the level of oxidative stress in the body (Kannan and Jain, 2000).

The cell membrane is the site most frequently attacked by free radicals. Oxidation or destruction of lipid membrane components by free radicals causes changes in the structure and function of cell membranes. Excess production of hydroxy radicals in organisms is a toxic factor that can damage the defense system responsible for eliminating these radicals, causing lipid peroxidation. It leads to several diseases such as cancer, neurodegeneration, and heart disease (Bonarska-Kujawa et al., 2014).

Superoxide dismutase is the most critical protective enzyme for the elimination of superoxide anion free radicals in various tissues and organs. It can transform highly reactive O_2 (oxygen) to low reactive H_2O_2 (hydrogen peroxide) and keep O_2 at a certain level in the body (Nagami et al., 2005). Catalase (CAT) is an iron-containing enzyme mainly found in different tissues including red blood cells to decompose H_2O_2 and eliminate toxic effect (Kumerova et al., 1998). Glutathione peroxidase, a selenium-containing enzyme widely distributed in the body, can promote the conversion of harmful substances produced in the lipid peroxidation reaction into corresponding

alcohols and block the chain cycle reaction. It can also decompose H_2O_2 into H_2O (water) (Yang et al., 2010).

In addition to the antioxidant enzyme system, there is an antioxidant non-enzyme system which includes vitamin C, vitamin E, glutathione, carotenoid, and the microelements copper, zinc, selenium, and manganese (Karami et al., 2018). These non-enzyme substances participate in biotransformation in the body. Most of the non-enzymatic substances are obtained from feed intake. Vitamin E is an essential antioxidant in biological systems and can penetrate into the lipid bilayer structure to combine with vitamin C and other antioxidant systems to terminate lipid oxidation (Reed, 2011).

Oxidative stress is defined as the imbalance between oxidants and antioxidants potentially leading to cell damage. A particularly destructive aspect of oxidative stress is the excessive production of reactive oxygen species (ROS), such as free radicals and peroxides, that cannot be effectively neutralized by the body (Delosière et al., 2013; Zhong and Zhou, 2013). In homeostasis, ROS are deactivated by endogenous antioxidants represented by enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidants (uric acid, glutathione, coenzyme Q) (Zhong and Zhou, 2013).

2. Oxidative stress and its effect on the animal and their product

During the production period, animals are exposed to a variety of factors that can increase oxidative stress, including diet, environment, and management. Diet factors include fat level and type of fatty acids, antioxidant content in the feed, storage, mycotoxin contamination, and hypervitaminosis, while environmental factors include temperature, humidity, dust, ammonia, radiation, hyperoxia, and bacterial or viral exposure. These conditions can promote the formation of free radicals and increase oxidative stress (Panda and Cherian, 2014).

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the capacity of cells to produce antioxidants. The balance between the production and neutralization of ROS with antioxidants is important. Free radicals attack important macromolecules, causing cell damage and disrupting homeostasis. Free radicals attack all types of molecules in the body,

including DNA, proteins, carbohydrates, and lipids (Lobo et al., 2010). This condition adversely affects poultry productive performance due to the oxidative stress causing apoptosis in follicular cells and then reducing the number of follicles and egg production (Li et al., 2020). In addition, oxidative stress also affects egg quality, yolk lipid, and cholesterol content (Scanes, 2016). Free radicals impact negatively feed intake, digestion, and absorption of nutrients by damaging the intestinal mucosa due to oxidation so that nutrients digestion and absorption are disrupted (Yara et al., 2013).

Oxidative stress is also linked to inflammation because oxidants or free radicals activate NF-kB, the major regulator of inflammation (Pantano et al., 2006). In an inactive state, it is bound to inhibitory proteins in the cytosol. Cytokines, bacterial stimuli, viruses, UV radiation, and oxidants cause inhibitory proteins to detach from nuclear factor-kappa B (NF-kB), translocate into the nucleus, and activate transcription of a number of genes involved in all aspects of inflammation (Gessner et al., 2017). Proinflammatory cytokines, chemokines, inflammatory enzymes, adhesion molecules, and some receptors are typical proteins encoded by NF-kB target genes (Hiscott et al., 1993; Aggarwal, 2004). NF-kB-regulated proteins, such as cytokines and chemokines stimulate oxidant generation by activated neutrophils (respiratory burst) and within mitochondria, thereby increasing oxidative stress (Pillarisetti and Saxena, 2004).

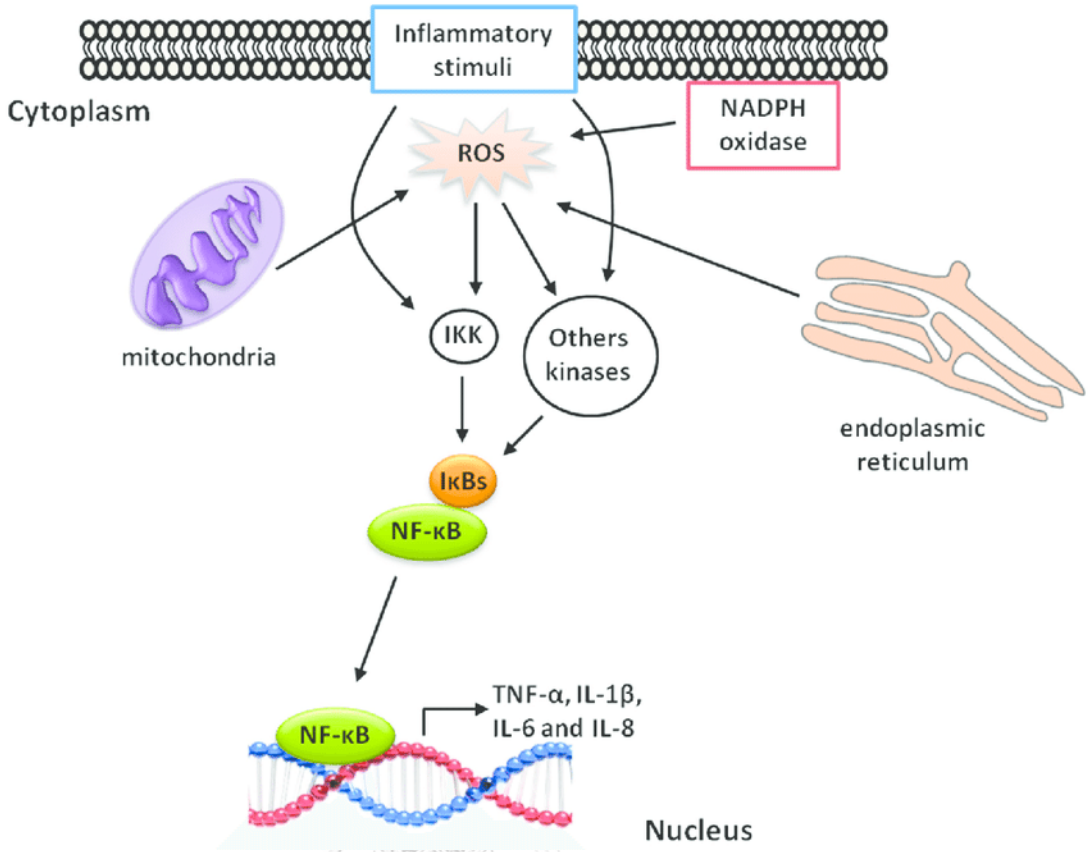


Figure 1 ROS-mediated nuclear factor kappa-B (NF-B) activation signaling pathways (Minatel et al., 2016)

Inflammation increases the production of ROS in cells, either through NADPH oxidase or the mitochondrial electron transport chain. These reactive molecules are directly related to the progress of the inflammatory process because they induce cell damage which triggers the activation of redox-sensitive transcription factors (Oka et al., 2010). Among the transcription factors, NF-kB plays a role in regulating proinflammatory genes, which represents a key step in the production of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) , and interleukins (IL-1β, IL-6, and IL-8) (Gilmore, 2006). The activity of NF-kB is regulated by a protein inhibitor (IkB). Inflammatory stimuli or ROS produced by mitochondria, NADPH oxidase, and endoplasmic reticulum trigger kinase pathways that activate NF-kB. After being released from its inhibitory protein (IkB), NF-kB is translocated to the cell nucleus and induces target gene transcription such as TNF-α, IL-1β, IL-6, and IL-8 (Minatel et al., 2016).

3. Antioxidant agent to alleviate oxidative stress

Antioxidants are compounds that delay, prevent, or eliminate oxidative damage to target molecules by scavenging or inhibiting the formation of reactive oxygen species (ROS) (Halliwell, 2007; Khlebnikov et al., 2007). The antioxidant system is divided into two major groups, enzymes and non-enzymes. The major enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GRx), and peroxiredoxins while non-enzymatic antioxidants are vitamins, glutathione, uric acid, lipoic acid, bilirubin, etc. (Carocho and Ferreira, 2013). The most effective antioxidant enzymes are GSH-Px, CAT, and SOD. GSH-Px and SOD are found in the mitochondria and cytosol, while CAT is produced in peroxisomes. SOD converts superoxide into H_2O_2 and oxygen, while GSH-Px and catalase react with H_2O_2 to produce water and oxygen (Rodriguez et al., 2004). Ascorbate, fibrin, glutathione, melatonin, mycothiol, phenolics, and serum albumin are non-enzymatic antioxidant molecules (Irato and Santovito, 2021). Non-enzymatic substances involved in the first line of defense are preventive antioxidants represented by their ability to rapidly inactivate radicals and oxidants (Mirończuk-Chodakowska et al., 2018). The antioxidant defense system prevents cell damage caused by reactive oxygen species (ROS) during exposure to infection, inflammation, and stress. Antioxidants prevent lipid peroxidation by blocking the peroxidation chain reaction and acting as ROS scavengers (Melhem et al., 2005; Valko et al., 2007).

Antioxidants can neutralize free radicals, and their effect occurs in stages. They act at three levels: prevention, interception, and repair. Preventive antioxidants, such as superoxide dismutase (SOD), catalyze the dismutation of superoxide to H_2O_2, which is then broken down by catalase into water. Free radical interception is primarily accomplished through radical scavenging, with secondary levels involving peroxyl radical scavenging. Antioxidants such as vitamins C and E, glutathione, other thiol compounds, carotenoids, flavonoids, and others are examples of effectors. At the level of repair and recovery, mainly repair enzymes are involved (Auroma et al., 1993).

4. Vitamin E as antioxidant

The commercial poultry industry is associated with stress which adversely affects animal health and production. These stress factors include environment, technology,

nutrition, and internal physiology which are responsible for decreasing poultry production and performance. At the molecular level, oxidative stress is associated with damage to biological molecules. Poultry feed contains many antioxidant supplements and one of them is vitamin E which is considered as a potential antioxidant. Supplementation of selenium, vitamin E, and carotenoids can modulate antioxidant defenses in poultry (Surai and Kochish, 2019). Vitamin E can protect cell membranes from oxidative damage (Van Acker et al., 1993).

Vitamin E (α-tocopherol) is a fat-soluble vitamin that acts as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles such as low-density lipoprotein (LDL). Vitamin E is able to intercept lipid peroxyl radicals (LOO*) and to stop the lipid peroxidation chain reaction (Nimse and Pal, 2015). Vitamin E is an important fat-soluble nutrient. Its role in animal production is indispensable because animals are unable to synthesize vitamin E (Shakeri et al., 2020). Vitamin E supplementation is reported to have benefits as an antioxidant, anti-inflammatory, and fertility (Traber, 2007; Panda et al., 2008; Ipek and Dikmen, 2014). According to the recommendations of the National Research Council (Council, 1994), vitamin E supplementation for laying hens should be 5-10 IU/kg. However, in the modern poultry industry, the addition of vitamin E is 25 IU/kg due to avoid unexpected conditions such as ambient temperature changes, nutrition imbalance, and inflammatory challenges (Jiang et al., 2013; Liu et al., 2019). However, the vitamin E supplementation at higher concentration than the NRC recommendations did not give any adverse effects on laying hens (Sünder and Flachowsky, 2001).

Vitamin E supplementation significantly increases egg production and quality by facilitating the release of egg yolk precursor (vitellogenin) from the liver, which also acts as an anti-stress (Ciftci et al., 2005). Antioxidants such as tocopherols can be added to laying hens' diets to protect fatty acids from oxidation and enrich eggs with vitamin E. Vitamin E is one of the key components in the antioxidant system that can reduce lipid peroxidation, prevent oxidation of eggs, and increase shelf life thereby increasing consumer acceptance (Galobart et al., 2001; Asadi et al., 2017). Previous studies have shown that antioxidants in the diet are transferred into the egg, thereby preventing the oxidation of egg fatty acids (Botsoglou et al., 2005; Ahmad et al., 2015).

5. The utilization of polyphenols as natural antioxidants in animals

The dietary intake of antioxidants is important to reduce oxidative damage in the body (Halliwell, 1996). These antioxidants play a role in protecting cellular components from the negative effect of ROS, thereby maintaining cellular function (Nimalaratne and Wu, 2015). Several synthetic antioxidants such as butylated hydroxy-anisole (BHA), butylated hydroxytoluene (BHT), and tetrabutyl hydroquinone (TBHQ) are widely used as feed additives to prevent oxidative damage. These compounds are reported to be toxic and carcinogenic (Prior, 2004; Vandghanooni et al., 2013). Therefore, the utilization of natural antioxidants is important to reduce consumers' negative perceptions of synthetic antioxidants.

The interest in natural antioxidants has been growing and being explored. The majority of natural antioxidants are phenolic compounds, and the most important groups of natural antioxidants are tocopherols, flavonoids, and phenolic acids (Gülcin, 2012). Polyphenols is a secondary plant metabolites that are commonly found in human and animal diets. They are found in a variety of plant parts, including roots, leaves, flowers, fruit, and seeds, and they protect plants from pests and UV radiation. Polyphenols contain active ingredients, have non-specific effects on living organisms, and regulate enzyme and cell receptor activity (D Archivio et al., 2007). Polyphenols are potent antioxidants that protect against oxidative stress (Paszkiewicz et al., 2012). Flavonoids are the most abundant type of polyphenol, and they are further classified into six subclasses: anthocyanins, isoflavones, flavanones, flavonols, flavones, and flavanols (Surai, 2014).

Bioflavonoids found in fruits and vegetables have been shown to have a variety of biological impacts, including free radical scavenging action. Bioflavonoids have been shown to protect against DNA damage induced by hydroxyl radicals. Metal ions, such as copper or iron, can be chelated by bioflavonoids. Biofavonoid complexes with copper or iron inhibit ROS production (Nimse and Pal, 2015). Antioxidant properties of flavonoids are depending on structural features such as the number and position of hydroxyl groups and the number of phenol rings. Flavonoids can scavenge peroxyl radicals, prevent lipid peroxidation, and chelate metal ions (Duthie and Crozier, 2000; Rice-Evans, 2001; Procházková et al., 2011). Flavonoids are also effective

supplementary agents to improve antioxidant status, enhance the immune system, and increase antioxidant enzyme activity to scavenge oxygen free radicals leading to reduced malondialdehyde concentration (Goliomytis et al., 2014).

Quercetin belongs to the flavanol class in the flavonoid group which is abundant in fruits and vegetables (Arabbi et al., 2004). Quercetin is a potent antioxidant that protects against diseases related to oxidative stress such as inflammation, cancer, heart disease, and degenerative diseases (McDermott, 2000). Liu et al. (2014) reported that supplementation of quercetin at 0.2 and 0.4 g/kg diet significantly increased laying rate and feed efficiency. The groups with the quercetin supplementation also have a higher value in Haugh unit, eggshell strength, eggshell thickness, and yolk protein compared to the control. The addition of onion extract rich in quercetin 0.0032% in hen diets increased egg weight, albumen, and yolk quality (Damaziak et al., 2017). Quercetin supplementation at 0.4 g/kg of feed in laying hens increased feed intake and eggshell weight compared to its concentrations at 0.2 and 0.8 g/kg feed. The quercetin at all levels in the diet also improved egg yolk oxidative stability of the fresh egg and stored egg for up to 90 days (Simitzis et al., 2018). Gopi et al. (2020) found that the addition of 0.05 g/kg of polyphenol extract from pomegranate peel increased the productivity of a broiler fed with broken-rice sorghum as a source of cereal under stress conditions. In a study conducted by Wang et al. (2017) tea polyphenols at doses of 0.6 or 1 g/kg of feed increased egg production and albumen quality in the late-laying phase. Zhu et al. (2020) found that the addition of different tea polyphenols (epigallocatechin gallate, DL-catechin, epigallocatechin, epicatechin, gallate, epigallocatechin, catechin) at concentration of 1 g/kg diet reduced the average feed intake and increased the feed efficiency of laying hens in the first 4 weeks. The use of resveratrol 0.4 g/kg in quail improved the antioxidant status of both birds and eggs (Sahin et al., 2010).

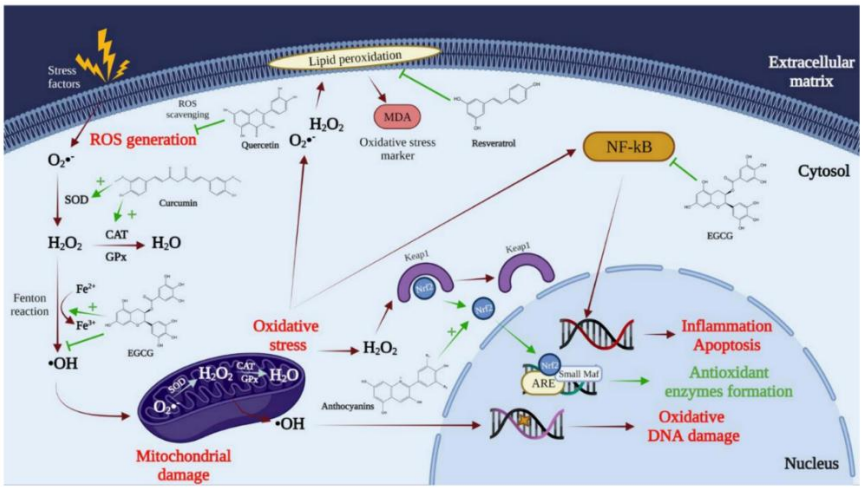


Figure 2 The mechanisms by which polyphenols interact with free radicals to reduce oxidative damage

(Enaru et al., 2021)

Polyphenols are substances that have antioxidant effects that can neutralize free radicals by donating an electron or a hydrogen atom. Polyphenols can inhibit the formation of free radicals thereby reducing the rate of oxidation by inhibiting the formation of reactive species and free radical precursors or by deactivating them (Enaru et al., 2021). According to Tsao (2010), polyphenols can directly act as radical scavengers (chain breakers) in the lipid peroxidation chain by transferring electrons to free radicals, causing them to become stable or less reactive, and subsequently stop the reaction. Furthermore, polyphenols can function as metal chelating agents in the Fenton reaction thereby reducing the rate of reaction and preventing the formation of hydroxyl radicals (Pietta, 2000; Perron and Brumaghim, 2009). Polyphenols also act as co-antioxidants and are part of the process of regenerating essential vitamins so that these compounds will increase the activity of endogenous antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase (Zhou et al., 2005; Du et al., 2007). Furthermore, several polyphenolic substances can stimulate the release of Nrf2, a transcription factor for oxidative stress defense systems. When cells are subjected to oxidative stress, the bond between Nrf2 and Keap1 breaks, causing Nrf2 to be released and accumulate in the cytoplasm. After that, it is easily translocated into the nucleus to play its role as a transcription factor gene expression to create antioxidant enzymes.

6. Bioavailabilty and metabolism of polyphenol

The absorption of nutrients into the body for their utilization for metabolic function is defined as bioavailability (Palafox-Carlos et al., 2011). Polyphenols are bioactive compounds that are neither macro nor micronutrients but have benefits and effects on specific cells and tissues. The availability of polyphenols depends on the type of compound, the physical and chemical properties, and the presence of functional groups (D Archivio et al., 2007).

In poultry or monogastric animals, the metabolism of phenolic compounds begins in the upper intestinal epithelium and then proceeds to the lower intestine, liver, and then in peripheral tissues, including adipose tissue and kidney. The route of absorption of phenolic compounds can be through the stomach and intestine or absorbed in the colon after chemical modification by microbiota in the colon. Microbes in the colon allows polyphenols to be absorbed into the bloodstream and then excreted through bile or urine (Brenes et al., 2016). Only 5 to 10% of the polyphenols consumed are absorbed by the small intestine, while 90-95% will pass to the large intestine along with other conjugates excreted by bile (Chiva-Blanch and Visioli, 2012). Subsequently, polyphenols are exposed to intestinal enzymes and gut microbes, which degrade the polyphenolic structure into smaller molecules. In the animal body, polyphenols are transformed into glycosides, esters, and polymeric forms which need to be hydrolyzed by gut microbes and intestinal enzymes for absorption.

Phenolic compounds with a less complex structure will undergo several biotransformations namely oxidation, reduction, and hydrolysis which convert them into water-soluble metabolites in enterocytes before reaching the liver. Complex phenolic compounds are not absorbed in the small intestine and reach the colon. In the colon, the gut microbiota will hydrolyze glycosides to form aglycones. This process reduces the complexity of the phenolic hydroxyl group structure to a phenol metabolite with a low molecular weight that can be absorbed. Once absorbed, these molecules will enter the liver via hepatocytes, where they will undergo a biotransformation process called conjugation which facilitates the absorption (the hydrophobicity) of the molecules and aids in rapid elimination. Finally, metabolites

enter the circulation system and are distributed to target organs or excreted through the urine (Cardona et al., 2013; Lavefve et al., 2020).

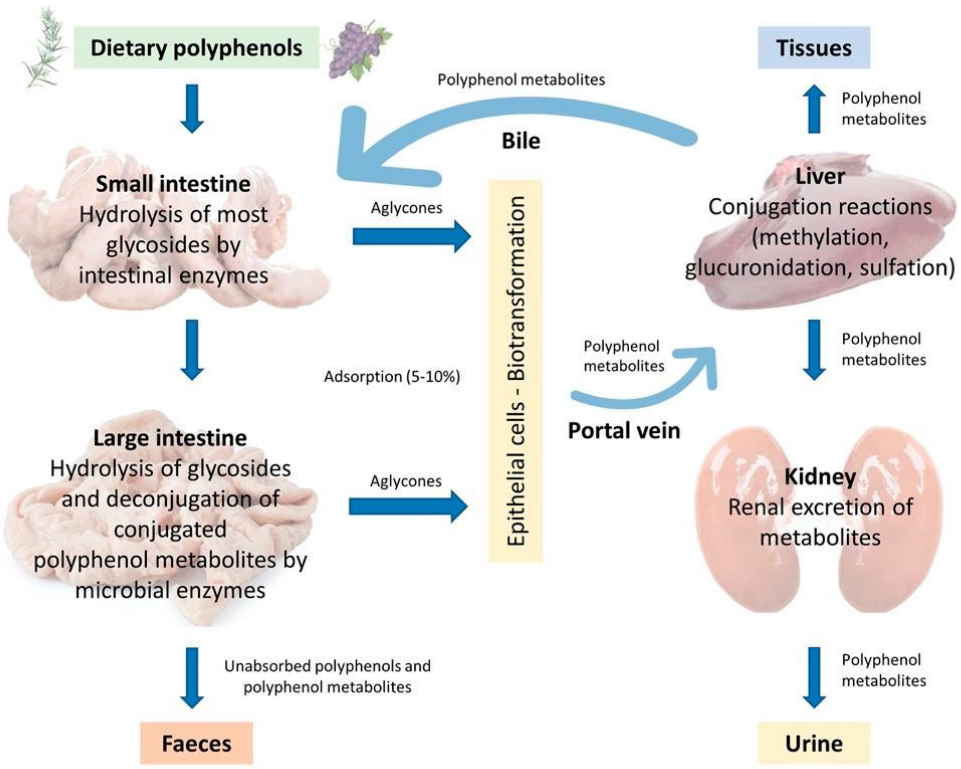


Figure 3 Plant polyphenol absorption and metabolism in monogastric farm animals (Serra et al., 2021)

7. *Boesenbergia rotunda* application in animal

B. rotunda, known as fingerroot, is a plant from the Zingiberaceae family. *B. rotunda* is distributed in Southeast Asia such as Indonesia, Malaysia, and Thailand (Abdelwahab et al., 2011). It is popular to be used as a medicine or as a cooking condiment. *B. rotunda* contains many compounds that have medicinal potential and are widely used as a traditional medicine for rheumatism, muscle pain, pain relief, gastric disorder, and flatulence (Tan et al., 2011; Eng-Chong et al., 2012).

Intensive research on this plant has been widely investigated in terms of phytochemical analysis, and biological evaluation. The major phytochemicals of this plant are flavonoids (e.g. flavanones, flavones, chalcones, and prenylated flavonoids) which have biological potential due to their medical effects (Chahyadi et al., 2014). Bioactive compounds of *B. rotunda* are grouped into two main groups, namely

flavanones (e.g. alpinetin, pinostrobin, and pinocembrin) and chalcones (e.g. boesenbergin, cardamonin, panduratin A, and 4-hydroxypanduratin A) (Kirana et al., 2007; Jing et al., 2010; Yusuf et al., 2013).

Pinostrobin is reported to increase the activity of antioxidant enzymes and quinone reductase (Fahey and Stephenson, 2002). Pinostrobin significantly decreased malondialdehyde concentration in the stomach of rats induced gastric ulcers with ethanol and could stimulate the mucosal defense system by stimulating gastric mucosal secretion and scavenging reactive oxygen species (super-oxide anions) and free radicals (Abdelwahab et al., 2011). The rhizome extract of *B. rotunda* showed biological activity as an antioxidant, reduced hepatic lipid accumulation, and might prevent non-alcoholic fatty liver disease (NAFLD) (Kiat et al., 2006; Kim et al., 2012). The ethanol extract, bioactive compounds, and flavanones (pinocembrin and pinostrobin) from *B. rotunda* showed antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Tanjung et al., 2013; Atun et al., 2018). The ethanol extract of *B. rotunda* had an antioxidant activity with a DPPH value of 76.3 mg/mL (Jitvaropas et al., 2012). Boesenbergin A, one of the major active compounds of *B. rotunda* showed antioxidant potential based on the Oxygen Radical Antioxidant Capacity (ORAC) assay. Boesenbergin A 20 µg/mL is equivalent to a Trolox concentration of 11.91 µM (*water-soluble derivative of vitamin E with potent antioxidant properties)* (Isa et al., 2012).

Previous studies on *B. rotunda* as a feed supplement either in powder or extract have been conducted. The oil extract of fingerroot at 356 ppm in broilers diet had the potential for anticoccidiocidal activity as salinomycin at 60 ppm, promoted feed intake and had no toxicity to animal tissues even after prolonged administration (Jitviriyanon et al., 2016). The supplementation of fingerroot powder up to 20 g/kg diet increased the protein mass of broiler meat, protein digestibility, nitrogen retention, feed intake, body weight, carcass percentage, and meat-bone ratio, but decreased feed conversion ratio (Artanto et al., 2016; Astungkarawati et al., 2016; Mentari et al., 2016). Supplementation of fingerroot powder at the level of 10 g/kg diet in Nile tilapia fish improved growth performance, increased skin mucus activity as well as serum immune mechanisms, and provided resistance to pathogenic bacteria *Streptococcus agalactiae* (Van Doan et al., 2019). The inclusion of a 4 g/kg diet of *B. rotunda* extract has a strong

effect on the improvement of growth, feed efficiency, immunity, and resistance against infection by the bacteria *Aeromonas hydrophila* and *Pseudomonas fluorescens* in the Goldfish (Hardi et al., 2021). Therefore, the supplementation of *B rotunda* in laying hens' diet is interesting for further research, especially on their production performance, egg quality, and egg yolk oxidative stability.

CHAPTER 3
MATERIALS AND METHODS

1. *Fingerroot powder preparation and analysis*

A total of 150 kg of fresh rhizomes of fingerroot were purchased from a local market in Nakhon Pathom province, Thailand. The fingerroot was cleaned, thinly sliced, and dried in an oven at 60 °C for 24 hours. The dry fingerroot rhizomes were powdered following the method by Abdelwahab *et al.* (2011). The total phenolic and total flavonoid content of fingerroot powder was determined by using the Folin-Ciocalteu assay and aluminum chloride assay. Total phenolic content was expressed as mg gallic acid equivalents (GAE) in mg 100 mg of powder, while the total flavonoid content was expressed as mg quercetin equivalents (QE). The proximate analysis of fingerroot powder was carried out out by the methods of the Association of Official Analytical Chemists (AOAC, 2000).

Table 1 The total flavonoid and phenolic compound of fingerroot powder

Compound Test	Result
Total phenolics	0.72 (GAE mg/100 mg)
Total flavonoids	0.62 (QE mg/ 100 mg)

Table 2 Proximate composition of fingerroot powder

Item	Amount
Gross energy (Cal/g)	3997.70
Crude protein (%)	6.21
Crude fibre (%)	5.71
Fat (%)	2.21
Moisture (%)	3.72
Ash (%)	7.66

2. *Birds, diets, and management*

A total of 192 Hy-Line brown laying hens aged 43 weeks were divided into four experimental groups, with 48 hens each (8 replicates; 6 birds). Laying hens were placed in a metal wire cage (40 cm width × 40 length x 30 height) with three birds per cage in the evaporative cooling system house. The average temperature and humidity levels were around 30 °C and 88%, respectively. Throughout the trial, daily lighting of 16 hours of light and 8 hours of darkness was used. The feed and water were provided ad libitum. The experimental diets were formulated by using the corn and soybean meal basal diet in mash form (Table 3) according to the nutritional recommendation of the Hy-Line brown laying hen described in the management guide (2018). Dietary treatments were as follows: control (T0) (basal diet), a basal diet supplemented with vitamin E at 250 mg/kg diet as positive control (T1), a basal diet containing fingerroot at 20 (T2) and 40 (T3) g/kg diet, respectively. Table 3 shows the ingredients and the nutrient composition of experimental diets. The experimental period of feeding trial was conducted for 42 days.

Table 3 Ingredients and nutrients composition of the experimental diet

Item	Experimental diets[1]			
	T0	T1	T2	T3
Ingredient				
Corn	59.54	59.54	57.33	55.11
Soybean meal	21.63	21.63	21.71	21.79
FFSB	5.00	5.00	5.00	5.00
Soybean oil	1.79	1.79	1.92	2.05
DCP	1.70	1.70	1.71	1.72
Limestone	9.51	9.51	9.50	9.50
Salt	0.40	0.40	0.40	0.40
DL-Met	0.18	0.18	0.18	0.18
Premix[2]	0.25	0.25	0.25	0.25
Vitamin E	-	0.025	-	-
Fingerroot powder	-	-	2.00	4.00
Calculated energy and nutrient content				
ME (kcal/kg)	2800	2800	2800	2800
Crude protein (%)	16.00	16.00	16.00	16.00
Lysine (%)	0.78	0.78	0.77	0.77
Methionine (%)	0.41	0.41	0.41	0.41
Fat (%)	5.11	5.11	5.2	5.31
Fiber (%)	3.07	3.07	3.14	3.21
Ca (%)	3.91	3.91	3.91	3.91
Available P (%)	0.40	0.40	0.40	0.40

[1]T0: control diet; T1: supplemented with vitamin E at 250 mg/kg diet; T2: basal diet containing fingerroot at 20 g/kg diet; T3: basal diet containing fingerroot at 40 g/kg diet

[2]Premix composition per kg feed: Vitamin A = 5,000 IU, Vitamin D3 = 800 IU, Vitamin E = 5 mg, Vitamin K3 = 0.825 mg, Vitamin B1 = 0.55 mg, Vitamin B2 = 1.125 mg, Vitamin B12 = 0.011 mg, Nicotinic acid = 1.5 mg, Copper = 0.25 mg, Iron = 25 mg, Iodine = 0.375 mg, Mn = 22 mg, Cobalt = 0.325 mg, Ca = 132 mg.

3. Sample collection and analysis

3.1 Productive performance

The parameters of laying performance include feed intake, egg production, egg weight, egg mass and feed conversion ratio. The parameters of laying performance were monitored during the experimental period. Feeding, egg collection, and data recording was carried out every day.

The feed residues were weighted weekly to calculate the feed intake. The feed intake was calculated by the formula according to (Sung and Adeola, 2022):

Feed intake (g/bird/day) = amount of feed consumed (g) ÷ the number of bird. (1)

The eggs were weighted daily and the average of the egg weight was obtained weekly. The egg production and egg mass were determined by these following formula (Araújo et al., 2015):

Egg production (%) = (the number of produced egg ÷ number of bird) x 100. (2)

Egg mass (g) = egg production (%) x egg weight (g). (3)

The feed conversion ratio was calculated by the formula according to Clark et al. (2019):

FCR = feed intake (g) ÷ egg mass production (g). (4)

3.2 Egg quality and composition

In the last of 3rd and 6th week of the experiment, 2 eggs per replicate were selected randomly for egg quality tests (16 eggs per group/ 64 eggs in total). The internal and external of egg quality parameters evaluated included egg weight, eggshell strength, eggshell thickness, egg shape index, Haugh unit, yolk color, yolk index, albumen height, whole egg composition (albumen weight, yolk weight, shell weight) and proportion (yolk-to-egg weight ratio, albumen-to-egg weight ratio, eggshell-to-egg weight ratio). Egg weight, eggshell strength, albumin height, Haugh unit, yolk index and yolk color were determined by using a Digital Egg Tester DET6500 (Nabel, Kyoto, Japan). The yolk index was calculated by dividing the yolk height by the yolk diameter. The yolk was weighed separately to obtain its weight and its ratio to egg weight. The albumen weight is obtained by subtracting egg weight from yolk weight and shell weight. The eggshells

were rinsed and air-dried at room temperature overnight and shell weight were recorded. The dried egghells were used to evaluate the shell thickness.

The eggshell thickness and shape index are important physical properties because they influence crushing strength and cracked egg risk. The thickness of dried shells with the membrane still intact was measured with a micrometer to the nearest 0.1 mm. The measurements were taken at three points on the eggshell: around the equator, the blunt, and the sharp area of the eggshell. The mean of the three locations was used to determined the shell thickness. The egg shape index was determined by measuring the egg length and width with a caliper. The egg shape index value was calculated by using the following formula according to (Anderson et al., 2004):

Shape Index = diameter/height x 100. (4)

The eggs shape index values was classified into three groups based on the shape index value obtained, namely sharp eggs (SI<72), normal eggs (SI=72-76), and round eggs (SI>76).

3.3 Lipid peroxidation of egg yolk

Lipid peroxidation is a process that occurs when free radicals react with polyunsaturated fatty acids in cell membranes, resulting in the formation of lipid peroxidation products, such as malondialdehyde. Malondialdehyde is biomarker that widely to assess lipid peroxidation. On the last day of the experiment, a total of 128 eggs (4 eggs per replicate) were randomly collected for storage at room temperature, and the malondialdehyde of the egg yolk was evaluated on the 0, 7, 14, and 21st days after storage.

The malondialdehyde measurement was conducted by the TBARS method as described by Kara et al. (2016). About 1 g of egg yolk was diluted in 9 ml of 1.15% KCl. 0.1 ml of this dillution was homogenized in 5 mL of 80 mM Trismaleate buffer (pH 7.4), 0.2 mL of 5 mM iron sulfate, and 0.2 mL of 2 mM ascorbic acid at 3000 rpm for 10 seconds in capped tubes on a vortex. The 2 mL of TCA-TBA-HCl solution (150 g of trichloroacetic acid and 3.75 g of thiobarbituric acid dissolved in 1 L of 0.25 N HCl) was

added to the homogenate in the tubes. The tubes were incubated at 100 ℃ for 15 minutes. After that, the tubes were centrifuged for 20 minutes at 2200 rpm (+4℃). A spectrophotometer was used to evaluate the absorbance of the supernatant at 535 nm in comparison to a blank. The concentration of malondialdehyde in analyzed samples (nmol/mg, yolk) will be calculated using the formula according to Kara et al. (2016):

MDA (nmol/mg) = (6.4102 × 1000 × 3 × absorbance value) ÷ (100 × mg/mL samples). (6)

4. Data analysis

Statistical analysis of data were performed with the SAS program by using one-way ANOVA for performance and egg quality, two-way ANOVA for egg yolk malondialdehyde, followed by Duncan's post hoc test. All values are expressed as the mean±standard deviation (SD) of eight replicates, and the data are considered statistically significant only if the p-values are less than 0.05.

CHAPTER 4
RESULTS

1. Productive performance

The influence of experimental diets on productive performance is shown in Table 4. There was no significant different in egg weight, egg mass, laying rate, feed intake, and feed conversion ratio during the first experimental period (1 to 3 week). In the second period of the experiment (4 to 6 week), there was a significant difference in egg weight and feed intake. The egg weight of the layers fed diet containing 20 g fingerroot powder (T2) was higher than the control group ($P < 0.05$). There was a significant decrease ($P < 0.05$) in feed intake in the group fed diet containing 40 g fingerroot powder (T3) compared to other groups. During 1^{st}-6^{th} week, feed intake in T3 was lower than that in T1 and T2 ($P < 0.05$) but it was not different from T1. Moreover, the inclusion of 40 g fingerroot powder to the layer diet had better FCR compared to the others ($P < 0.05$) during the second and in the whole experimental period.

Table 4 The effect of fingerroot powder on the laying performance

Parameter	Treatments[1]				SEM[2]	P-Value
	T0	T1	T2	T3		
Feed intake (g/h/d)						
1-3 week	119.22	123.06	121.91	117.31	5.18	0.1345
4-6 week	108.30^{b}	110.28^{b}	109.91^{b}	102.34^{a}	4.12	0.0020*
1-6 week	113.76^{ab}	116.67^{b}	115.91^{b}	109.83^{a}	4.11	0.0113*
Laying rate (%)						
1-3 week	91.66	93.55	89.91	92.16	3.38	0.2153
4-6 week	92.15	93.32	87.41	91.83	5.10	0.1279
1-6 week	91.91	93.44	88.65	91.99	3.80	0.1046

Egg weight (g)						
1-3 week	60.57	61.44	61.90	61.47	1.40	0.3069
4-6 week	60.30[a]	61.09[ab]	62.26[b]	61.66[ab]	1.33	0.0404*
1-6 week	60.43	61.26	62.08	61.57	1.33	0.1142
Egg mass (g)						
1-3 week	55.54	57.49	55.66	56.67	2.59	0.4061
4-6 week	55.57	57.03	54.51	56.65	3.75	0.3792
1-6 week	55.55	57.26	55.09	56.66	2.97	0.4569
Feed conversion ratio						
1-3 week	2.15	2.15	2.19	2.08	0.009	0.1032
4-6 week	1.96[b]	1.94[b]	2.03[b]	1.81[a]	0.111	0.0041*
1-6 week	2.05[b]	2.04[b]	2.11[b]	1.94[a]	0.007	0.0009*

Different letters in the same row (a, b, c) indicate significant differences ($P < 0.05$).

[1]T0: control diet; T1: supplemented with vitamin E at 250 mg/kg diet; T2: basal diet containing fingerroot at 20 g/kg diet; T3: basal diet containing fingerroot at 40 g/kg diet. [2]SEM = standard error of the mean.

2. Egg quality

The influences of experimental diets on internal egg quality are summarized in **Table 5.** At 3 weeks of feeding, no difference was found in all parameters except for the egg yolk color. The yolk color in T3 was significantly different from that in T0 and T1 ($P < 0.05$) but it was not different from that of T2. However, at 6 weeks of feeding, the experimental diets did not give any different effect on internal egg quality.

The physical characteristics of the egg were measured to determine the external egg quality. The data of external egg quality is shown in **Table 6**. The shell strength, shell thickness, and shape index were not influenced by the experimental diets among groups in both experimental periods.

The experimental diets gave results with significant differences in the albumin weight and shell weight at 6 weeks of feeding (**Table 7**). The diet supplemented with fingerroot powder at 20 g (T2) had a higher albumen weight and shell weight compared

to the control (T0) and vitamin E (T1) diets. However, there was not significant difference between T2 and T3.

The proportion of the whole egg composition is presented in **Table 8**. The data indicates that the experimental diets did not affect all egg proportion parameters as no significant difference was found among groups.

Table 5 The effect of fingerroot powder on internal egg quality

Parameter	Treatments[1]				SEM[2]	P-Value
	T0	T1	T2	T3		
At the end of 3 weeks						
Albumen height (mm)	8.33	8.45	8.14	8.18	0.68	0.7882
Haugh unit	90.35	90.91	88.95	88.59	3.44	0.6866
Yolk index	0.441	0.443	0.431	0.432	0.003	0.7850
Yolk color	5.85^{b}	5.70^{b}	5.60^{ab}	5.34^{a}	0.26	0.0049*
At the end of 6 weeks						
Albumen height (mm)	7.79	7.40	8.02	8.15	0.84	0.3168
Haugh unit	86.85	86.98	88.52	89.84	6.39	0.7599
Yolk index	0.437	0.432	0.448	0.433	0.02	0.5848
Yolk color	5.21	5.11	5.09	4.84	0.43	0.4179

Different letters in the same row (a, b, c) indicate significant differences ($P < 0.05$).

[1]T0: control diet; T1: supplemented with vitamin E at 250 mg/kg diet; T2: basal diet containing fingerroot at 20 g/kg diet; T3: basal diet containing fingerroot at 40 g/kg diet. [2]SEM = standard error of the mean.

Table 6 The effect of fingerroot powder on external egg quality

Parameter	Treatments[1]				SEM[2]	P-Value
	T0	T1	T2	T3		
At the end of 3 weeks						
Shell strength (N)	51.33	49.55	51.33	49.90	5.88	0.8932
Shell thickness (mm)	0.38	0.37	0.40	0.39	0.018	0.0936
Shape index	92.84	93.00	93.02	92.83	0.53	0.8443
At the end of 6 weeks						
Shell strength (N)	44.65	46.62	45.78	46.97	3.53	0.5676
Shell thickness (mm)	0.36	0.36	0.38	0.37	0.017	0.3994
Shape index	93.21	92.57	93.19	93.14	0.56	0.0950

Different letters in the same row (a, b, c) indicate significant differences ($P < 0.05$).

[1]T0: control diet; T1: supplemented with vitamin E at 250 mg/kg diet; T2: basal diet containing fingerroot at 20 g/kg diet; T3: basal diet containing fingerroot at 40 g/kg diet.

[2]SEM = standard error of the mean.

Table 7 The effect of fingerroot powder on the whole egg composition

Parameter	Treatments[1]				SEM[2]	P-Value
	T0	T1	T2	T3		
At the end of 3 weeks						
Albumen weight (g)	42.09	41.88	42.91	41.65	3.47	0.8970
Yolk weight (g)	15.54	15.73	15.49	15.32	0.94	0.8568
Shell weight (g)	6.30	6.06	6.64	5.92	0.69	0.2015
At the end of 6 weeks						
Albumen weight (g)	38.87[a]	38.63[a]	41.67[b]	40.04[ab]	2.06	0.0237*
Yolk weight (g)	15.01	14.81	14.87	14.99	0.66	0.9219
Shell weight (g)	5.77[a]	5.78[a]	6.24[b]	5.93[ab]	0.34	0.0330*

Different letters in the same row (a, b, c) indicate significant differences ($P < 0.05$).

[1]T0: control diet; T1: supplemented with vitamin E at 250 mg/kg diet; T2: basal diet containing fingerroot at 20 g/kg diet; T3: basal diet containing fingerroot at 40 g/kg diet. [2]SEM = standard error of the mean.

Table 8 The effect of fingerroot powder on the proportion of whole egg (%)

Parameter	Treatments[1]				SEM[2]	P-Value
	T0	T1	T2	T3		
At the end of 3 weeks						
Albumen ratio	65.80	65.71	65.91	66.14	1.89	0.9721
Yolk ratio	24.39	24.75	23.88	24.42	1.44	0.6879
Shell ratio	9.82	9.53	10.20	9.44	0.96	0.4011
At the end of 6 weeks						
Albumen ratio	65.37	64.95	66.35	65.53	1.02	0.0724
Yolk ratio	26.05	25.30	23.70	24.69	2.05	0.1571
Shell ratio	9.69	9.74	9.95	9.77	0.39	0.6064

Different letters in the same row (a, b, c) indicate significant differences ($P < 0.05$).

[1]T0: control diet; T1: supplemented with vitamin E at 250 mg/kg diet; T2: basal diet containing fingerroot at 20 g/kg diet; T3: basal diet containing fingerroot at 40 g/kg diet.

[2]SEM = standard error of the mean.

3. Lipid peroxidation of the egg yolk

The effect of fingerroot powder supplementation on egg lipid peroxidation was evaluated by measuring malondialdehyde levels in fresh and stored eggs at 7, 14, and 21 days. The results showed that the experimental diets did not affect malondialdehyde levels in fresh or stored eggs. The malondialdehyde levels in egg yolk were increased in all groups at 21 days of storage. The diets did not impact on the malondialdehyde level in the egg yolks. The malondialdehyde levels in the egg yolk increased by storage time. There was no relationship between the diet and storage time.

Table 9 Malondialdehyde level of fresh and stored egg

		MDA level (nmol/mg)
Main effect		
Diet	T0	1.196±0.032
	T1	1.134±0.031
	T2	1.188±0.029
	T3	1.173±0.030
Storage time	0	1.022 ±0.030[a]
	7	1.098±0.032[a]
	14	1.017±0.029[a]
	21	1.555±0.032[b]
Diet		0.503
Storage time		0.000
Diet*Storage time		0.616

Superscript letters indicate significant differences ($P < 0.05$).

CHAPTER 5
DISCUSSION

1. Laying hen performance

The addition of fingerroot powder at 40 g/kg diet decreased feed intake. Based on its total polyphenol, fingerroot powder at concentrations of 20 and 40 g contained 268 and 536 mg of polyphenol respectively. According to Darmawan et al. (2022) the optimum dose of phytogenic feed additives for laying hens is 300 mg/kg of diet. Higher level of phytogenic additives tend to reduce production performance and feed efficiency. The relatively high polyphenol content in the diet may be responsible for the decrease in feed intake at a concentration of 40 g. This study is similar to Ghasemi et al. (2010) who found that the feed intake of layers was decreased during 6 weeks of garlic and thymes powder supplementation. According to Brenes and Roura (2010), the inclusion of phytogenic compounds in poultry decreased feed palatability due to pungent odor, leading to reduce feed intake. However, the study found that 40 g fingerroot did not reduce production performance. In agreement with our findings, Sahin et al., (2010) observed that the laying performance parameters were unaffected by the addition of 200 and 400 ppm resveratrol. Several studies reported that supplementation of phytogenic feed additives such as garlic, fennel, and coneflower provided benefits in increasing production performance such as increased body weight, feed conversion, egg production and quality in both broilers and laying hens (Aji et al., 2011; Khan et al., 2011; Rahimi et al., 2011).

It was discovered that adding fingerroot powder at a concentration of 20 g improved the average egg weight during the second experimental period. However, the egg mass did not affected by the experimental diets. The polyphenol compounds in fingerroot may be involved in hormonal regulation that plays a role in egg formation. According to Jing et al. (2010), fingerroot contains polyphenol compounds such as caffeic acid, coumaric acid, chlorogenic acid, hesperidin, kaempferol, naringin, and quercetin. Kaempferol is a bioflavonoid with several beneficial properties, including anticancer, anti-inflammatory, and antioxidant effects (Luo et al., 2011). Flavonoids have a comparable structure to steroid hormones, particularly estrogens (Zand et al.,

2002). Some flavonoids have a similar structure to 17β-estradiol. 17β-estradiol is the major estrogen involved in laying hen sexual maturation, as well as the activation of egg shell development and egg interior components (Hanlon et al., 2022).

It was observed that fingerroot powder at concentartion 40 g improved feed efficiency. Lower feed conversion value indicates that the feed utilized efficiently. Wang et al. (2017) stated that phytogenic feed additives can increase the secretion and activity of the enzymes amylase, trypsin, chymotrypsin, and lipase. Boka et al. (2014) also reported that phytogenic feed additives contribute in modulating gut microbes, enhancing digestibility and absorption of nutrients, and improving ovarian health that subsequently improves laying performance (Boka et al., 2014).

Improvement in nutrient digestibility may also be related to morphological changes in the gut such as modifications in the size of villi and crypts in the jejunum and colon in chickens that ingest phytogenic feed additives. Adibmorabi et al. (2006) reported that garlic powder supplementation increased villi height and decreased crypt depth. Longer villi increase the surface area resulting in the increase of available nutrient absorption (Yasar, 1999). According to some studies, the increases in feed efficiency in poultry fed phenolic compounds was due to changes in the intestinal surface area as well as the activity of digestive enzymes, which lead to better nutrient absorption (Mountzouris et al., 2011; Viveros et al., 2011).

Although many studies report that phytogenic feed additives may improve animal performance, some also report the opposite. This is due to many factors such as location or plant origin, plant species, growth stage, harvest time, soil type, climate and stress conditions, cultivation practices, fertilization, and irrigation (Yitbarek, 2015). The different results of phytogenic feed additives may be due to the use of different phytogenic products, the level and time of inclusion, and the interaction of the compounds from each plant (Saki et al., 2014).

Excessive polyphenols had a detrimental effect on digestive tract health, nutrient absorption, digestive enzyme activity, vitamin and mineral absorption, laying performance, and egg quality (Abd El-Hack et al., 2023). The presence of polyphenol compounds in diet may have several adverse metabolic effects related to lower

nutritional efficiency, inhibition of digestive enzymes, and increased excretion of endogenous proteins (Brenes and Roura, 2010). Polyphenols have a detrimental impact on poultry when highly added in the diet. The decline in poultry performance after polyphenols supplementation can be attributed to lower fat and protein digestibility via two mechanisms i.e, polyphenols binding to bile salts or deactivated digestive enzymes (Brenes and Roura, 2010). According to Krogdahl (1985), the presence of tannins in some polyphenols could bind bile salts thereby reducing fat digestibility. Several studies confirmed that polyphenols could inhibit digestive enzymes such as α-glucosidase, α-amylase, lipase, and trypsin (Longstaff and McNb, 1991; You et al., 2011;Yilmazer-Musa et al., 2012). Harigome et al. (1988) also stated that polyphenols reduced the activity of digestive enzymes and could bind to feed ingredients and endogenous proteins to form insoluble complexes. Some polyphenol compounds also reduced the absorption of minerals such as iron, zinc, and copper (MA, 2013). However, no negative effects were found from fingerrot powder supplementation in this study.

2. Egg quality

2.1 Albumen quality

Our finding suggest that fingerroot at 20 g/kg diet improved albumen weight. Fingerroot contains many active compounds that has anti-inflammatory and antioxidant properties such as pinocembrin, pinostrombin, alpinetin, panduratin, cardamonin, quercetin, and kaempferol (Eng-Chong et al., 2012). These active ingredients may be able to minimize oxidative stress, prevent cell damage, and improve the health of the magnum that responsible for the synthesis and secretion of albumen. Saraswati et al. (2014) found an increase in albumen height and egg protein in layers-fed turmeric powder, demonstrating that the active ingredients in turmeric powder improved the proliferation of epithelial cells and tubular gland cells in the magnum, allowing them to synthesize and produce albumen. According to Saraswati et al. (2013) and Herve et al. (2019) the success of phytogenic supplementation of turmeric powder and essential oil of ginger is mediated by increasing the concentration of circulating estrogen, which is the main reproductive hormone that plays a role in determining egg mass and quality. Estrogen hormone not only stimulates the production of yolk material that includes very low-density lipoprotein (VLDL),

vitellogenin, apolipoprotein (apo) B, and apoVLDL-II in the liver but also controls the oviduct in synthesizing and secreting albumen (El-Azeem et al., 2018).

The increased albumin weight in this study also could be due to the polyphenols in the fingerroot influencing the protein albumen. Wang et al. (2018) also found that adding tea polyphenols to laying hen's diet increased ovomucin fraction in albumen.

The experimental diets in both experimental periods did not affect on albumen height and Haugh unit. Our finding is similar to previous research conducted by Yalcin et al. (2006) who found that feeding 5 and 10 g/kg garlic powder had no influence on albumen index and Haugh unit values in laying hens. Ghasemi et al. (2010) reported that Haugh unit was unaffected by garlic supplementation at 2 g/kg diet.

One of the primary protein components of albumin is ovomucin, which helps to maintain the structure of the protein by stabilizing protein-protein bonds, the nature of the viscous gel, and the height of albumen thickness, all of which contribute to the Haugh unit value (Omana et al., 2010; Wang et al., 2018). Albumen quality is assessed by using the Haugh unit (HU) value; a high HU value indicates thick albumen with a strong viscosity, which reflects superior albumen quality (Zhang et al., 2020b). Albumen qualiy depends on the amount of β-ovomucin secreted by the magnum, and β-ovomucin is responsible for albumen height (Ozgan et al., 2009). Ovomucin is the factor responsible for albumen height. The deterioration of ovomucin caused a decrease in albumin height (Stevens, 1996).

Several studies reported that Haugh unit score increased in laying hens supplemented with phytogenic feed additives (Abdel-Wareth and Lohakare, 2020; Marume et al., 2020). According to the United States Department of Agriculture (USDA) (2000), eggs labeled grade AA (excellent quality) must have a HU value of more than 72. Meanwhile, eggs with HU values between 55 and 72 are defined as grade A (high-quality eggs), eggs with HU values greater than 30 are classified as grade B (medium-quality eggs), and eggs with HU values less than 30 are classified as low-quality eggs. However, the Haugh unit value in this study ranged from 88.59 to 90.91, indicating that the eggs were of good quality and classified as grade AA.

2.2 Egg yolk quality

The experimental diet did not affect egg yolk weight and yolk index. The yolk weight in this study ranged from 14.81 to 15.73 g, with its percentage to egg weight of 23.70-26.05%. The yolk index ranges from 0.432 to 0.448. The previous study reported that the supplementation of tea polyphenol at 600 mg/kg diet has no effect on yolk index (Zhou et al., 2020). Another stuy also stated that no effect found on yolk index in laying hens fed with grape seed (Kara et al., 2016). The yolk index is the ratio between the height and the diameter of the yolk. The yolk index describes the freshness of the eggs. Fresh eggs have a yolk index value ranging from 0.33-0.50, with an average of 0.42. Increasing the shelf life of eggs reduces the yolk index because the size of the yolk increases due to water passing from the albumen (Swacita and Cipta, 2011).

The supplementation of fingerroot powder at a concentration of 40 g significantly decreased egg yolk color at 3 weeks feeding. The possible explanation is that the feed supplemented with 40 g fingerroot powder contains less corn (Table 1) containing zeaxanthin, which is responsible for the color of egg yolks. Egg yolk color is affected by the consumption of zeaxanthin, lutein, alpha-carotene, beta-carotene, and carotenoids (Hammershøj et al., 2010). The lower feed intake in this group might affect egg yolk color. The possible reason is because the lower feed intake impacts the delivery of nutrients, particularly pigments. Inadequate pigment intake appears to be the cause of the decrease in egg yolk color as compared to the control and vitamin E-supplemented group. Laying hens are unable to synthesize the pigment responsible for yolk color. The pigment that deposited in egg yolk are hydroxy carotenoids or xanthophylls that is dependent on the fat-soluble pigment present in the diet (Nelson and Baptist, 1968; Yildirim et al., 2013).

Vitamin E is a natural antioxidant that affects stabilizing egg yolk color when added to poultry feed . The egg yolk pigment is associated with fat molecules in the yolk membrane, in which vitamin E can stabilize egg yolk pigment (Jackson et al., 1978). However, vitamin E supplementation at a level of 250 mg/kg of feed in this study did not show any difference from the control on egg yolk color.

2.3 Egg shell quality

The physical characteristic of animal products is essential because it influences the treatment and equipment used during transportation, processing, packaging, and storage. In the case of eggs, the shell must be strong to prevent the egg damage. The mechanical characteristics of egg quality include shape index, shell thickness, and shell strength.

The quality of the shell in this study was evaluated by measuring several variables including shell weight, shell ratio, shell strength, shell thickness, and shape index. In this study, all experimental diets did not affect shell quality variables except the shell weight. Layers fed with 20 g fingerroot powder had the highest shell weight compared to other groups. It may be related to the effect of polyphenols on oestrogen. Liu et al (2013) stated that quercetin increases eggshell weight because it has an estrogen-like action that plays a role in calcium metabolism. Bolukbasi (2008) reported that the supplementation with 200 mg/kg of thyme, sage, or rosemary essential oil increased the proportion of eggshells. However, another study showed no effect of supplementation of black cumin essential oil on shell weight (Bölükbaşi et al., 2009). The supplementation of plant additives showed positive effect on external and internal quality of eggs.. A possible mechanism behind this positive effect is that phytogenics have the ability to improve uterine health and increase calcium storage as well as pancreatic secretion, resulting in improved egg and eggshell quality (Abdel-Wareth and Lohakare, 2020).

There were no differences found in shell ratio, shell thickness, and shell strength among the experimental diets. The shell ratio of the study ranged from 9.53 to 10.20% of the egg weight, the shell thickness ranged from 0.36 to 0.40 mm and shell strength ranged between 44.65 and 51.33 N. Some studies reported that the supplementation of plant-derived powder or extract gives various results. According to (Kaya et al., 2013), supplementation of the mixtures of a plant extract containing extracts of *Origanum vulgare*, *Thymus vulgaris*, and oils of thyme, origanum, garlic, anise, and fennel at different concentrations improved eggshell thickness. In contrast, Botsoglou et al. (2005) demonstrated that the supplementation of rosemary, oregano, and saffron in the laying hen's diet had no effect on eggshell thickness. Magnolol supplementation

significantly reduced shell thickness but did not affect shell strength (Chen et al., 2021). External egg quality is varied among species because eggshell formation is a complex process depending on several mechanisms which can be influenced by genetics, environmental conditions, strain, age of the bird, nutrition, and management.

Several studies have found that polyphenols influence calcium bioavailability and absorption. The major tea flavonoid, Epigallocatechin gallate (EGCG) can induce apoptosis in osteoclasts, decrease bone resorption, and enhance mineral density (Muraki et al., 2007). However, other polyphenol such as caffeine and tannins, can enhance calcium excretion through urine, decrease calcium bioavailability and absorption in the gut (Huang et al., 2016; Almaraj and Pius, 2017). Calcium uptake reduced due to high caffeine in the gastrointestinal system (Hallstro et al., 2006).

The shell quality is influenced by age, nutrition, health state, and environment. When the bird age increases, the shell quality decreases, whereas egg weight increases (Machal and Simeinovava, 2002). The average shell thickness of a bird age at 57 weeks was 0.373 mm, which is thinner than that (0.403) of the bird at age 22 weeks (Al-Batshan et al., 1994). A diet with low amino acids, linoleic acids, and oil was employed to suppress egg weight in order to maintain shell quality (Petersen et al., 1983). The dietary calcium also affected shell quality. The NRC (1994) recommend that a daily calcium intake of bird is 3.25 g per day. Calcium intake in aged birds can be increased to maintain shell quality (Bar et al., 2002). Calcium and bicarbonate ions must be delivered via the blood to the oviduct and transported into the uterine wall during egg calcification (Nys et al., 2004). The carbonic anhydrase in the cytoplasm of uterine epithelial cells is involved in shell formation. This enzyme continuously converts carbonic acid into carbon dioxide gas, enabling carbonate ions to be pumped through the cell membrane (Nys et al., 2004).

The shell is a unique structure that functions to protect the internal egg from mechanical damage and invasion of microorganisms. Shell thickness and shell structure are important aspects of egg quality because they affect the egg's resistance to damage. Eggs with thicker shells are more resistant to bacterial penetration and can reduce the risk of cracking. Shell thickness is related to egg weight. The heavier the egg, the thinner the shell thickness, and this affects water loss during storage

(Giampauli et al., 2005). The strength of the shell is affected by its microstructure, thickness, mass, volume, surface area, and shell percentage (Nedomova et al., 2009).

The shape index did not affected by the diets. The shape index value was above 90. According to Altuntas and Sekeroglu (2008) shape index is divided into three classes based on the width-to-length ratio of the egg: sharp, normal, and round eggs with the value of each is 72, 72–76, and >76 respectively. Shape index is an indicator of egg quality. According to Shaker et al. (2017), the shape index is influenced by strain of bird and environmental factors. The shape index strongly correlated with breed and declines with age (Van den Brand et al., 2004; Camp et al., 2007).

3. Lipid peroxidation of the egg yolk

After seven days of storage under room temperature (25 °C), egg quality was significantly impaired compared to fresh eggs, since stored eggs were unable to maintain their chemical-physical composition (Alleoni and Antunes, 2001). The use of natural antioxidant products has been considered an interesting approach to improve egg quality and animal health. In this study, the use of fingerroot powder supplementation to laying hens diet were evaluated to improve egg shelf life quality. The study found that the experimental diets had no effect on the malondialdehyde levels in fresh and stored eggs at 7, 14, and 21 days. The malondialdehyde level in egg yolk increased after stored 21 days. The malondialdehyde level in the egg yolk did not affected by the diets but increased by the storage time. The fingerroot powder at 20 and 40 g, as well as vitamin E supplementation at 250 mg/kg did not exert their antioxidant activity on the egg yolk in terms of reducing lipid peroxidation.

Some previous studies reported the effect of herbal supplementation or phytogenic additives on lipid peroxidation in egg yolks. Curcumin, the main biactive compound of turmeric was reported to able to maintain egg quality after 21 days of storage (Galli et al., 2018). Kaya et al. (2013) also found that essential oils and vitamin E can improve egg quality by lowering oxidant levels. Kara et al. (2016) also reported that grape pomace supplementation significantly reduced lipid peroxidation in egg yolk. An et al. (2019) reported that the administration of lycopene at concentrations of 10 and 20 mg and 17 g of tomato paste reduced the MDA in eggs stored for 4 weeks at 24 °C. Lycopene and carotenoids, two of the bioactive components in tomatoes,

can be effectively transferred into the egg yolk (Akdemir et al., 2012). Moreover, the supplementation of tomato pomace increased the amount of lycopene and lutein in egg yolks (Reda et al., 2022).

Many studies have reported that bioactive compounds in the diet may be transferred into egg yolks. The most widely studied phytochemical compounds in egg yolk are lutein and zeaxanthin (Nimalaratne et al., 2011). Kuhnle et al. (2008) also discovered that isoflavones derived from soybean was present in egg yolk. In the current study, the supplementation of fingerroot powder to laying hens did not give any effect on malondialdehyde of the egg yolk. This possible reason is that the active compounds in fingerroot cannot be transferred into the egg yolk. Polyphenols are reported to have different characteristics such as their hydrophilic which affect their ability to cross cell membranes. In addition, it was reported that under natural conditions, the deposition of phenolic compounds in chicken egg yolks was very limited (Nimalaratne et al., 2011).

The total polyphenol in the fingerroot powder at doses of 20 and 40 g were 268 and 536 mg respectively. Phenolic compounds in the diet may also decrease due to storage time and environmental changes. Phenolic compounds are unstable and easily degraded during storage, affecting their biological activity (Srivastava et al., 2007). De Oliveira et al. (2017) discovered that phenolic compounds in sorghum grains decreased during 60-180 days of storage, with a decrease from 89.4% to 100%. According to (Radovanović et al., 2017), the decrease in phenolic levels is also influenced by several factors such as temperature, oxygen, light, and storage time. High temperatures, light, and the presence of oxygen will oxidize phenolic compounds due to unsaturated bonds in their molecular structure. The storage at ± 45 °C has the effect of reducing phenolic compounds (Zhang et al., 2000). At high storage temperatures, the possibility of phenolic compounds being damaged is high because the opportunity for oxidation is greater (Rodríguez-Pérez et al., 2019). Light can reduce the antioxidant activity of phenolic compounds because the main component of light is UV (Ultra Violet) which has oxidative properties to react with oxygen to form photo-oxidation or light-induced oxidation reactions. UV radiation causes a homolytic fission reaction of hydrogen peroxide (H_2O_2) to become a highly reactive hydroxyl free radical (OH*). In addition, UV

radiation can also trigger the formation of reactive oxygen species (ROS) (Mahardani and Yuanita, 2021).

CONCLUSION

At six weeks of feeding, the fingerroot powder at a level of 20 g improved egg weight, albumen weight, and shell weight. Meanwhile, the fingerroot powder inclusion at level of 40 g decreased egg yolk color and feed intake but improved feed efficiency. The fingerroot powder did not show antioxidant activity in terms of MDA in both fresh and stored eggs. The results of this study suggested that fingerroot powder at 20-40 g can be added in the laying hens' diet.

REFERENCES

Abd El-Hack ME, Salem HM, Khafaga AF, Soliman SM and El-Saadony MT 2023. Impacts of polyphenols on laying hens' productivity and egg quality: A review. Journal of Animal Physiology and Animal Nutrition. 107: 928-947.

Abdel-Wareth A and Lohakare J 2020. Productive performance, egg quality, nutrients digestibility, and physiological response of bovans brown hens fed various dietary inclusion levels of peppermint oil. Animal Feed Science and Technology. 267: 114554.

Abdelwahab SI, Mohan S, Abdulla MA, Sukari MA, Abdul AB, Taha MME, Syam S, Ahmad S and Lee K-H 2011. The methanolic extract of *Boesenbergia rotunda* (L.) Mansf. and its major compound pinostrobin induces anti-ulcerogenic property in vivo: possible involvement of indirect antioxidant action. Journal of ethnopharmacology. 137: 963-970.

Aggarwal BB 2004. Nuclear factor-**K**B: the enemy within. Cancer cell. 6: 203-208.

Ahmad S, Gokulakrishnan P, Giriprasad R and Yatoo M 2015. Fruit-based natural antioxidants in meat and meat products: A review. Critical reviews in food science and nutrition. 55: 1503-1513.

Aji SB, Ignatius K, Ado A, Nuhu JB, Abdulkarim A, Aliyu U, Gambo MB, Ibrahim MA, Abubakar H and Bukar M 2011. Effect of feeding onion (Allium cepa) and garlic (Allium sativum) on some performance characteristics of broiler chickens. Res. J. Poult. Sci. 4: 22-27.

Akdemir F and Sahin K 2009. Genistein supplementation to the quail: effects on egg production and egg yolk genistein, daidzein, and lipid peroxidation levels. Poultry Science. 88: 2125-2131.

Akdemir F, Orhan C, Sahin N, Sahin K and Hayirli A 2012. Tomato powder in laying hen diets: effects on concentrations of yolk carotenoids and lipid peroxidation. British Poultry Science. 53: 675-680.

Amalraj A and Pius A 2017. Relative Contribution of Oxalic Acid, Phytate and Tannic Acid on the Bioavailability of Calcium from Various Calcium Salts-An in Vitro Study. Int. Food Res. J. 24: 1278–1285.

Alleoni ACC and Antunes AJ 2001. Haugh unit as a measure of the quality of hen eggs stored under refrigeration. Scientia Agricola. 58: 681-685.

Altuntaş E and Şekeroğlu A 2008. Effect of egg shape index on mechanical properties of chicken eggs. Journal of Food Engineering. 85: 606-612.

An B-K, Choo W-D, Kang C-W, Lee J and Lee K-W 2019. Effects of dietary lycopene or tomato paste on laying performance and serum lipids in laying hens and on malondialdehyde content in egg yolk upon storage. The Journal of Poultry Science. 56: 52-57.

Anderson K, Tharrington J, Curtis P and Jones F 2004. Shell characteristics of eggs from historic strains of single comb white leghorn chickens and the relationship of egg shape to shell strength. International journal of poultry science. 3: 17-19.

Arabbi PR, Genovese MI and Lajolo FM 2004. Flavonoids in vegetable foods commonly consumed in Brazil and estimated ingestion by the Brazilian population. Journal of agricultural and Food Chemistry. 52: 1124-1131.

Araújo W, Albino L, Rostagno H, Pessoa G, Cruz S, Lelis G, Carneiro P and Vieira R 2015. Sunflower meal and supplementation of an enzyme complex in layer diets. Brazilian Journal of Poultry Science. 17: 363-370.

Artanto ED, Sukamto B and Atmomarsono U 2016. The Effect of Feeding Fingerroot (*Boesenbergia Pandurata* Roxb.) Powder on Broiler Performance). Animal Agriculture Journal. 3: 89-94.

Asadi F, Shariatmadari F, Karimi-Torshizi M, Mohiti-Asli M and Ghanaatparast-Rashti M 2017. Comparison of different selenium sources and vitamin E in laying hen diet and their influences on egg selenium and cholesterol content, quality and oxidative stability. Iranian Journal of Applied Animal Science. 7: 83-89.

Astungkarawati D, Suthama N and Atmomarsono U 2016. Protein Utilization and Growth of Broiler Chicken Fed Dietary Finggeroot (*Boesenbergia Pandurata* Roxb.). Animal Agriculture Journal. 3: 163-171.

Atun S, Handayani S and Rakhmawati A 2018. Potential bioactive compounds isolated from *Boesenbergia rotunda* as antioxidant and antimicrobial agents. Pharmacognosy Journal. 10.

Auroma O, Murcia A, Butler J and Halliwell B 1993. Evaluation of the antioxidant and prooxidant action of gallic acid and its derivates. J. Agric. Food Chem. 41: 1880-1885.

Bar A, Razaphkovsky V, and Vax E. 2002. Re-evaluation of calcium and phosphorus requirements in aged laying hens. Br. Poult. Sci. 43: 261–269.

Batista N, Garcia E, Oliveira C, Arguelo N and Souza K 2017. Trace mineral sources and rosemary oil in the diet of brown laying hens: egg quality and lipid stability. Brazilian Journal of Poultry Science. 19: 663-672.

Boka J, Mahdavi A, Samie A and Jahanian R 2014. Effect of different levels of black cumin (N igella sativa L.) on performance, intestinal E scherichia coli colonization and jejunal morphology in laying hens. Journal of animal physiology and animal nutrition. 98: 373-383.

Bolukbasi SC 2008. The effect of feeding thyme, sage and rosemary oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and Escherichia coli count in feces. Archives fur Geflugelkunde. 72: 231-237.

Bölükbaşi ŞC, Kaynar O, Erhan MK and Urupan H 2009. Effect of feeding *Nigella sativa* oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and Escherichia coli count in feces. Archiv für Geflügelkunde. 73: 167-172.

Bonarska-Kujawa D, Pruchnik H, Cyboran S, Żyłka R, Oszmiański J and Kleszczyńska H 2014. Biophysical mechanism of the protective effect of blue honeysuckle (Lonicera caerulea L. var. kamtschatica Sevast.) polyphenols extracts against lipid peroxidation of erythrocyte and lipid membranes. The Journal of membrane biology. 247: 611-625.

Botsoglou N, Florou-Paneri P, Botsoglou E, Dotas V, Giannenas I, Koidis A and Mitrakos P 2005. The effect of feeding rosemary, oregano, saffron and α-tocopheryl acetate on hen performance and oxidative stability of eggs. South African Journal of Animal Science. 35: 143-151.

Brenes A and Roura E 2010. Essential oils in poultry nutrition: Main effects and modes of action. Animal feed science and technology. 158: 1-14.

Brenes A, Viveros A, Chamorro S and Arija I 2016. Use of polyphenol-rich grape by-products in monogastric nutrition. A review. Animal Feed Science and Technology. 211: 1-17.

Campo JL, Gil MG, and Dávila SG 2007. Differences among white-, tinted-, and brown-egg laying hens for incidence of eggs laid on the floor and for oviposition time. Archiv für Geflügelkunde. 71: 105–109

Cardona F, Andrés-Lacueva C, Tulipani S, Tinahones FJ and Queipo-Ortuño MI 2013. Benefits of polyphenols on gut microbiota and implications in human health. The Journal of nutritional biochemistry. 24: 1415-1422.

Carocho M and Ferreira IC 2013. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and chemical toxicology. 51: 15-25.

Chahyadi A, Hartati R and Wirasutisna KR 2014. *Boesenbergia pandurata* Roxb., an Indonesian medicinal plant: Phytochemistry, biological activity, plant biotechnology. Procedia Chemistry. 13: 13-37.

Chen Y, Chen H, Li W, Miao J, Chen N, Shao X and Cao Y 2018. Polyphenols in Eucalyptus leaves improved the egg and meat qualities and protected against ethanol‐induced oxidative damage in laying hens. Journal of Animal Physiology and Animal Nutrition. 102: 214-223.

Chiva-Blanch G and Visioli F 2012. Polyphenols and health: Moving beyond antioxidants. Journal of Berry Research. 2: 63-71.

Ciftci M, Ertas ON and Guler T 2005. Effects of vitamin E and vitamin C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress. Rev. Med. Vet. 156: 107-111.

Cimrin T, Avsaroglu M, Tunca RI, Kandir S and Ayasan T 2019. Effects of the dietary supplementation of layer diets with natural and synthetic antioxidant additives on yolk lipid peroxidation and fatty acid composition of eggs stored at different temperatures and duration. Brazilian Journal of Poultry Science. 21.

Council NR 1994. Nutrient requirements of poultry. National Academy of Sciences Washington.

D Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C and Masella R 2007. Polyphenols, dietary sources and bioavailability. Annali-Istituto Superiore di Sanita. 43: 348.

Damaziak K, Riedel J, Gozdowski D, Niemiec J, Siennicka A and Róg D 2017. Productive performance and egg quality of laying hens fed diets supplemented with garlic and onion extracts. Journal of Applied Poultry Research. 26: 337-349.

Darmawan A, Hermana W, Suci DM, Mutia R, Jayanegara A and Ozturk E 2022. Dietary Phytogenic Extracts Favorably Influence Productivity, Egg Quality, Blood Constituents, Antioxidant and Immunological Parameters of Laying Hens: A Meta-Analysis. Animals. 12: 2278.

de Oliveira KG, Queiroz VAV, de Almeida Carlos L, de Morais Cardoso L, Pinheiro-Sant'Ana HM, Anunciação PC, de Menezes CB, da Silva EC and Barros F 2017. Effect of the storage time and temperature on phenolic compounds of sorghum grain and flour. Food Chemistry. 216: 390-398.

Delosière M, Damon M and Durand D 2013. Oxidative stress in farm animals: General aspects. Cahiers de Nutrition et de Diététique. 48: 218-224.

Du Y, Guo H and Lou H 2007. Grape seed polyphenols protect cardiac cells from apoptosis via induction of endogenous antioxidant enzymes. Journal of agricultural and food chemistry. 55: 1695-1701.

Duthie G and Crozier A 2000. Plant-derived phenolic antioxidants. Current opinion in lipidology. 11: 43-47.

El-Azeem A, Nafisa A, Madkour M, Aboelazab O and El-Wardany I 2018. Productive performance and histological responses of japanese quail breeders to age at mating and silver nanoparticles administration. Egyptian Journal of Nutrition and Feeds. 21: 807-822.

Enaru B, Socaci S, Farcas A, Socaciu C, Danciu C, Stanila A and Diaconeasa Z 2021. Novel delivery systems of polyphenols and their potential health benefits. Pharmaceuticals. 14: 946.

Eng-Chong T, Yean-Kee L, Chin-Fei C, Choon-Han H, Sher-Ming W, Li-Ping CT, Gen-Teck F, Khalid N, Abd Rahman N and Karsani SA 2012. *Boesenbergia rotunda*: from

ethnomedicine to drug discovery. Evidence-Based Complementary and Alternative Medicine. 2012.

Fahey JW and Stephenson KK 2002. Pinostrobin from honey and Thai ginger (*Boesenbergia pandurata*): a potent flavonoid inducer of mammalian phase 2 chemoprotective and antioxidant enzymes. Journal of Agricultural and Food Chemistry. 50: 7472-7476.

Fascina VB, Pasquali GAM, Berto DA, Silva AdL, Garcia EA, Pezzato AC, Gonzales E and Sartori JR 2017. Effects of arginine and phytogenic additive supplementation on performance and health of brown-egg layers. Revista Brasileira de Zootecnia. 46: 502-514.

Florou-Paneri P, Giannenas I, Christaki E, Govaris A and Botsoglou N 2006. Performance of chickens and oxidative stability of the produced meat as affected by feed supplementation with oregano, vitamin C, vitamin E and their combinations. Archiv fur Geflugelkunde. 70: 232-239.

Galli GM, Da Silva AS, Biazus AH, Reis JH, Boiago MM, Topazio JP, Migliorini MJ, Guarda NS, Moresco RN and Ourique AF 2018. Feed addition of curcumin to laying hens showed anticoccidial effect, and improved egg quality and animal health. Research in veterinary science. 118: 101-106.

Galobart J, Barroeta A, Baucells M, Cortinas L and Guardiola F 2001. **α**-Tocopherol transfer efficiency and lipid oxidation in fresh and spray-dried eggs enriched with **ω**3-polyunsaturated fatty acids. Poultry science. 80: 1496-1505.

Gessner D, Ringseis R and Eder K 2017. Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. Journal of animal physiology and animal nutrition. 101: 605-628.

Ghasemi R, Zarei M and Torki M 2010. Adding medicinal herbs including garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) to diet of laying hens and evaluating productive performance and egg quality characteristics. Am J Anim Vet Sci. 5: 151-154.

Gilmore TD 2006. Introduction to NF-**K**B: players, pathways, perspectives. Oncogene. 25: 6680-6684.

Goliomytis M, Orfanou H, Petrou E, Charismiadou M, Simitzis P and Deligeorgis S 2014. Effect of hesperidin dietary supplementation on hen performance, egg quality and yolk oxidative stability. British Poultry Science. 55: 98-104.

Gopi M, Dutta N, Pattanaik AK, Jadhav SE, Madhupriya V, Tyagi PK and Mohan J 2020. Effect of polyphenol extract on performance, serum biochemistry, skin pigmentation and carcass characteristics in broiler chickens fed with different cereal sources under hot-humid conditions. Saudi Journal of Biological Sciences. 27: 2719-2726.

Gülcin I 2012. Antioxidant activity of food constituents: an overview. Archives of toxicology. 86: 345-391.

Halliwell B 1996. Antioxidants in human health and disease. Annual review of nutrition. 16: 33-50.

Halliwell B 2007. Biochemistry of oxidative stress. Biochemical society transactions. 35: 1147-1150.

Hammershøj M, Kidmose U and Steenfeldt S 2010. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (*Daucus carota*) varieties as forage material for egg-laying hens. Journal of the Science of Food and Agriculture. 90: 1163-1171.

Hardi EH, Saptiani G and Nugroho RA 2021. *Boesenbergia pandurata* application in Goldfish (Cyprinus carpio) Feed to Enhancing Fish Growth, Immunity System, and Resistance to Bacterial Infection. F1000Research. 10.

Herve T, Raphaël KJ, Ferdinand N, Victor Herman N, Willy Marvel NM, Cyril D'Alex T and Laurine Vitrice FT 2019. Effects of ginger (*Zingiber officinale*, Roscoe) essential oil on growth and laying performances, serum metabolites, and egg yolk antioxidant and cholesterol status in laying Japanese quail. Journal of veterinary medicine. 2019.

Hiscott J, Marois J, Garoufalis J, D'addario M, Roulston A, Kwan I, Pepin N, Lacoste J, Nguyen H and Bensi G 1993. Characterization of a functional NF-kappa B site in the human interleukin 1 beta promoter: evidence for a positive autoregulatory loop. Molecular and cellular biology. 13: 6231-6240.

Huang C and Tang R 2016. Tea Drinking Habits and Osteoporotic Hip/Femur Fractures: A Case-Control Study. Pakistan J. Med. Sci. 32: 408–412.

Ipek A and Dikmen BY 2014. The effects of vitamin E and vitamin C on sexual maturity body weight and hatching characteristics of Japanese quails (*Coturnix coturnix japonica*) reared under heat stress. Animal science papers and reports. 32: 261-268.

Irato P and Santovito G 2021. Enzymatic and non-enzymatic molecules with antioxidant function. Page 579 Vol. 10. MDPI.

Isa N, Abdelwahab S, Mohan S, Abdul A, Sukari M, Taha M, Syam S, Narrima P, Cheah SC and Ahmad S 2012. In vitro anti-inflammatory, cytotoxic and antioxidant activities of boesenbergin A, a chalcone isolated from *Boesenbergia rotunda* (L.)(fingerroot). Brazilian Journal of Medical and Biological Research. 45: 524-530.

Jackson D, Law G and Nockels C 1978. Maternal vitamin E alters passively acquired immunity of chicks. Poultry Science. 57: 70-73.

Jang A, Liu X-D, Shin M-H, Lee B-D, Lee S-K, Lee J-H and Jo C 2008. Antioxidative potential of raw breast meat from broiler chicks fed a dietary medicinal herb extract mix. Poultry science. 87: 2382-2389.

Jiang W, Zhang L and Shan A 2013. The effect of vitamin E on laying performance and egg quality in laying hens fed corn dried distillers grains with solubles. Poultry Science. 92: 2956-2964.

Jing LJ, Mohamed M, Rahmat A and Bakar MFA 2010. Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda, Boesenbergia pulchella* var attenuata and *Boesenbergia armeniaca*). Journal of Medicinal Plants Research. 4: 27-32.

Jitvaropas R, Saenthaweesuk S, Somparn N, Thuppia A, Sireeratawong S and Phoolcharoen W 2012. Antioxidant, antimicrobial and wound healing activities of *Boesenbergia rotunda*. Natural product communications. 7: 1934578X1200700727.

Jitviriyanon S, Phanthong P, Lomarat P, Bunyapraphatsara N, Porntrakulpipat S and Paraksa N 2016. In vitro study of anti-coccidial activity of essential oils from indigenous plants against *Eimeria tenella.* Veterinary parasitology. 228: 96-102.

Kannan K and Jain SK 2000. Oxidative stress and apoptosis. Pathophysiology. 7: 153-163.

Kara K, Kocaoğlu Güçlü B, Baytok E and Şentürk M 2016. Effects of grape pomace supplementation to laying hen diet on performance, egg quality, egg lipid peroxidation and some biochemical parameters. Journal of Applied Animal Research. 44: 303-310.

Karami M, Torki M and Mohammadi H 2018. Effects of dietary supplemental chromium methionine, zinc oxide, and ascorbic acid on performance, egg quality traits, and blood parameters of laying hens subjected to heat stress. Journal of applied animal research. 46: 1174-1184.

Kaya H, Kaya A, Celebi S and Macit M 2013. Effects of dietary supplementation of essential oils and vitamin E on performance, egg quality and Escherichia coli count in excreta. Indian Journal of Animal Research. 47: 515-520.

Khan RU, Nikousefat Z, Javdani M, Tufarelli V and Laudadio V 2011. Zinc-induced moulting: production and physiology. World's Poultry Science Journal. 67: 497-506.

Khlebnikov AI, Schepetkin IA, Domina NG, Kirpotina LN and Quinn MT 2007. Improved quantitative structure–activity relationship models to predict antioxidant activity of flavonoids in chemical, enzymatic, and cellular systems. Bioorganic & medicinal chemistry. 15: 1749-1770.

Kiat TS, Pippen R, Yusof R, Ibrahim H, Khalid N and Abd Rahman N 2006. Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, *Boesenbergia rotunda* (L.), towards dengue-2 virus NS3 protease. Bioorganic & medicinal chemistry letters. 16: 3337-3340.

Kim D-Y, Kim M-S, Sa B-K, Kim M-B and Hwang J-K 2012. *Boesenbergia pandurata* attenuates diet-induced obesity by activating AMP-activated protein kinase and regulating lipid metabolism. International journal of molecular sciences. 13: 994-1005.

Kirana C, Jones GP, Record IR and McIntosh GH 2007. Anticancer properties of panduratin A isolated from *Boesenbergia pandurata* (Zingiberaceae). Journal of Natural Medicines. 61: 131-137.

Krogdahl Å 1985. Digestion and absorption of lipids in poultry. The Journal of nutrition. 115: 675-685.

Kumerova A, Lece A, Skesters A, Silova A and Petuhovs V 1998. Anaemia and antioxidant defence of the red blood cells. Materia medica Polona. Polish journal of medicine and pharmacy. 30: 12-15.

Lavefve L, Howard LR and Carbonero F 2020. Berry polyphenols metabolism and impact on human gut microbiota and health. Food & function. 11: 45-65.

Li-Chan EC and Kim HO 2008. Structure and chemical composition of eggs. Egg bioscience and biotechnology. 10: 9780470181249.

Li G-M, Liu L-P, Yin B, Liu Y-Y, Dong W-W, Gong S, Zhang J and Tan J-H 2020. Heat stress decreases egg production of laying hens by inducing apoptosis of follicular cells via activating the FasL/Fas and TNF-α systems. Poultry science. 99: 6084-6093.

Lipiński K, Mazur M, Antoszkiewicz Z and Purwin C 2017. Polyphenols in monogastric nutrition–a review. Annals of Animal Science. 17: 41-58.

Liu W, Wang J, Zhang Z, Xu J, Xie Z, Slavin M and Gao X 2014. In vitro and in vivo antioxidant activity of a fructan from the roots of *Arctium lappa* L. International Journal of Biological Macromolecules. 65: 446-453.

Liu Y, Li Y, Liu H-N, Suo Y-L, Hu L-L, Feng X-A, Zhang L and Jin F 2013. Effect of quercetin on performance and egg quality during the late laying period of hens. British Poultry Science. 54: 510-514.

Liu Y, Zhao L, Mosenthin R, Zhang J, Ji C and Ma Q 2019. Protective effect of vitamin E on laying performance, antioxidant capacity, and immunity in laying hens challenged with *Salmonella enteritidis*. Poultry Science. 98: 5847-5854.

Lobo V, Patil A, Phatak A and Chandra N 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews. 4: 118.

Máchal L and Simeonovová J 2002. The relationship of shortening and strength of eggshell to some egg quality indicators and egg production in hens of different initial laying lines. Arch. Anim. Breed. 45: 287–296.

Mahardani OT and Yuanita L 2021. Efek Metode Pengolahan Dan Penyimpanan Terhadap Kadar Senyawa Fenolik Dan Aktivitas Antioksidan. UNESA Journal of Chemistry. 10: 64-78.

Marume U, Mokagane J, Shole C and Hugo A 2020. *Citrullus lanatus* essential oils inclusion in diets elicit nutraceutical effects on egg production, egg quality, and physiological characteristics in layer hens. Poultry science. 99: 3038-3046.

McDermott JH 2000. Antioxidant nutrients: current dietary recommendations and research update. Journal of the American Pharmaceutical Association (1996). 40: 785-799.

Melhem A, Stern M, Shibolet O, Israeli E, Ackerman Z, Pappo O, Hemed N, Rowe M, Ohana H and Zabrecky G 2005. Treatment of chronic hepatitis C virus infection via antioxidants: results of a phase I clinical trial. Journal of clinical gastroenterology. 39: 737-742.

Mentari AS, Mahfudz LD and Suthama N 2016. Protein and Fat Meat Mass in Broiler Chikens Which Given Finggerroot (*Boesenbergia Pandurata* Roxb.) Powder in Ration. Animal Agriculture Journal. 3: 211-220.

Minatel IO, Francisqueti FV, Corrêa CR and Lima GPP 2016. Antioxidant activity of γ-oryzanol: a complex network of interactions. International journal of molecular sciences. 17: 1107.

Mirończuk-Chodakowska I, Witkowska AM and Zujko ME 2018. Endogenous non-enzymatic antioxidants in the human body. Advances in medical sciences. 63: 68-78.

Mohamed AB, Huseen F and Jawad O 2011. Effect of Cinnamon (*Cinnamomum zylenicum*) supplementation on the intestinal selected bacterial population in Japanese quail. Research Opinions in Animal & Veterinary Sciences.

Muraki S, Yamamoto S, Ishibashi H, Oka H, Yoshimura N, Kawaguchi H, and Nakamura K 2007. Diet and Lifestyle Associated with Increased Bone Mineral Density: Cross-

Sectional Study of Japanese Elderly Women at an Osteoporosis Outpatient Clinic. J. Orthop. Sci. 12: 317–320.

Nagami H, Yoshimoto N, Umakoshi H, Shimanouchi T and Kuboi R 2005. Liposome-assisted activity of superoxide dismutase under oxidative stress. Journal of bioscience and bioengineering. 99: 423-428.

Nedomova S, Severa L and Buchar 2009. Influence of hen egg shape on eggshell compressive strength. International Agrophysics. 23: 249-256.

Nelson T and Baptist J 1968. Feed pigments: 2. The influence of feeding single and combined sources of red and yellow pigments on egg yolk color. Poultry Science. 47: 924-931.

Ngo D-H, Wijesekara I, Vo T-S, Van Ta Q and Kim S-K 2011. Marine food-derived functional ingredients as potential antioxidants in the food industry: An overview. Food Research International. 44: 523-529.

Nimalaratne C and Wu J 2015. Hen egg as an antioxidant food commodity: A review. Nutrients. 7: 8274-8293.

Nimse SB and Pal D 2015. Free radicals, natural antioxidants, and their reaction mechanisms. RSC advances. 5: 27986-28006.

Nys Y, Gautron J, Garcia Ruiz JM, and Hincke MT 2004. Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. C. R. Palevol. 3: 549–562.

Nys Y and Gautron J 2007. Structure and formation of the eggshell. Bioactive egg compounds. 99-102.

Nys Y, Zawadzki J, Gautron J and Mills A 1991. Whitening of brown-shelled eggs: mineral composition of uterine fluid and rate of protoporphyrin deposition. Poultry Science. 70: 1236-1245.

Oka T, Fujimoto M, Nagasaka R, Ushio H, Hori M and Ozaki H 2010. Cycloartenyl ferulate, a component of rice bran oil-derived γ-oryzanol, attenuates mast cell degranulation. Phytomedicine. 17: 152-156.

Omana DA, Wang J and Wu J 2010. Ovomucin–a glycoprotein with promising potential. Trends in food science & technology. 21: 455-463.

Palafox-Carlos H, Ayala-Zavala JF and González-Aguilar GA 2011. The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. Journal of food science. 76: R6-R15.

Panda A, Ramarao S, Raju M and Chatterjee R 2008. Effect of dietary supplementation with vitamins E and C on production performance, immune responses and antioxidant status of White Leghorn layers under tropical summer conditions. British Poultry Science. 49: 592-599.

Panda AK and Cherian G 2014. Role of vitamin E in counteracting oxidative stress in poultry. The Journal of Poultry Science. 51: 109-117.

Pantano C, Reynaert NL, Vliet AVD and Janssen–Heininger YM 2006. Redox-sensitive kinases of the nuclear factor-**K**B signaling pathway. Antioxidants & redox signaling. 8: 1791-1806.

Paszkiewicz M, Budzyńska A, Różalska B and Sadowska B 2012. The immunomodulatory role of plant polyphenols. Advances in Hygiene and Experimental Medicine. 66: 637-646.

Petersen CF, Sauter EA, Steele EE, and Parkinson JF 1983. Use of methionine intake restriction to improve egg shell quality by control of egg weight. Poultry Science. 62: 2044-2047.

Perron NR and Brumaghim JL 2009. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell biochemistry and biophysics. 53: 75-100.

Petti S and Scully C 2009. Polyphenols, oral health and disease: A review. Journal of dentistry. 37: 413-423.

Pietta P-G 2000. Flavonoids as antioxidants. Journal of natural products. 63: 1035-1042.

Pillarisetti S and Saxena U 2004. Role of oxidative stress and inflammation in the origin of Type 2 diabetes–a paradigm shift. Expert opinion on therapeutic targets. 8: 401-408.

Prior R 2004. Absorption and metabolism of anthocyanins: potential health effects. Phytochemicals: Mechanisms of action. 1-19.

Procházková D, Boušová I and Wilhelmová N 2011. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 82: 513-523.

Radovanović B, Radovanović A, Nikolić V, Manojlović N and Dimitrijević J 2017. Storage effect on phenolic content and antioxidant activity in selected fruit extracts. Bulg. Chem. Commun. 49: 879-883.

Rahimi S, Teymori Zadeh Z, Torshizi K, Omidbaigi R and Rokni H 2011. Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. Journal of Agricultural Science and Technology. 13: 527-539.

Ramalho VC and Jorge N 2006. Antioxidants used in oils, fats and fatty foods. Química Nova. 29: 755-760.

Reed TT 2011. Lipid peroxidation and neurodegenerative disease. Free Radical Biology and Medicine. 51: 1302-1319.

Rice-Evans C 2001. Flavonoid antioxidants. Current medicinal chemistry. 8: 797-807.

Rodríguez-Pérez C, Segura-Carretero A and del Mar Contreras M 2019. Phenolic compounds as natural and multifunctional anti-obesity agents: A review. Critical reviews in food science and nutrition. 59: 1212-1229.

Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V and Reiter RJ 2004. Regulation of antioxidant enzymes: a significant role for melatonin. Journal of pineal research. 36: 1-9.

Sahin K, Akdemir F, Orhan C, Tuzcu M, Hayirli A and Sahin N 2010. Effects of dietary resveratrol supplementation on egg production and antioxidant status. Poultry Science. 89: 1190-1198.

Saki AA, Aliarabi H, Siyar SAH, Salari J and Hashemi M 2014. Effect of a phytogenic feed additive on performance, ovarian morphology, serum lipid parameters and egg sensory quality in laying hen. Veterinary Research Forum:287.

Saraswati TR, Manalu W, Ekastuti DR, and Kusumorini N 2013. Increased egg production of Japanese quail (*Cortunix japonica*) by improving liver function through turmeric powder supplementation. International journal of poultry Science. 12: 601-614.

Saraswati TR, Manalu W, Ekastuti DR and Kusumorini N 2014. Effect of turmeric powder to estriol and progesterone hormone profile of laying hens during one cycle of ovulation. International Journal of Poultry Science. 13: 504.

Scanes CG 2016. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poultry science. 95: 2208-2215.

Schreiner M, Hulan H, Razzazi-Fazeli E, Böhm J and Iben C 2004. Feeding laying hens seal blubber oil: effects on egg yolk incorporation, stereospecific distribution of omega-3 fatty acids, and sensory aspects. Poultry Science. 83: 462-473.

Ser H-L, Tan LT-H, Palanisamy UD, Abd Malek SN, Yin W-F, Chan K-G, Goh B-H and Lee L-H 2016. *Streptomyces antioxidans* sp. nov., a novel mangrove soil actinobacterium with antioxidative and neuroprotective potentials. Frontiers in Microbiology. 7: 899.

Serra V, Salvatori G and Pastorelli G 2021. Dietary polyphenol supplementation in food producing animals: Effects on the quality of derived products. Animals. 11: 401.

Shakeri M, Oskoueian E, Le HH and Shakeri M 2020. Strategies to combat heat stress in broiler chickens: Unveiling the roles of selenium, vitamin E and vitamin C. Veterinary sciences. 7: 71.

Simitzis P, Spanou D, Glastra N and Goliomytis M 2018. Impact of dietary quercetin on laying hen performance, egg quality and yolk oxidative stability. Animal Feed Science and Technology. 239: 27-32.

Srivastava A, Akoh CC, Yi W, Fischer J and Krewer G 2007. Effect of storage conditions on the biological activity of phenolic compounds of blueberry extract packed in glass bottles. Journal of Agricultural and Food Chemistry. 55: 2705-2713.

Stevens L 1996. Egg proteins: what are their functions? Science Progress. 79: 65-87.

Sünder A and Flachowsky G 2001. Influence of high vitamin E dosages on retinol and carotinoid concentration in body tissues and eggs of laying hens. Archives of Animal Nutrition. 55: 43-52.

Sung JY and Adeola O 2022. Research Note: Estimation of individual feed intake of broiler chickens in group-housing systems. Poultry Science. 101: 101752.

Surai P 2014. Polyphenol compounds in the chicken/animal diet: from the past to the future. Journal of Animal Physiology and Animal Nutrition. 98: 19-31.

Surai PF and Kochish II 2019. Nutritional modulation of the antioxidant capacities in poultry: the case of selenium. Poultry science. 98: 4231-4239.

Swacita IBN and Cipta IS 2011. The Effect Of Farming System And Long Storage To Duck's Egg Quality. Buletin Veteriner Udayana. 3: 91-98.

Tan EC, Foo GT, Wong SM, Noorsaadah AR, Norzulaani K, Saiful AK, Shatrah O and Rohana Y 2011. Optimization of two-dimensional gel electrophoresis protocols for Boesenbergia rotunda in vitro suspension culture. Journal of Medicinal Plants Research. 5: 3777-3780.

Tanjung M, Tjahjandarie TS and Sentosa MH 2013. Antioxidant and cytotoxic agent from the rhizomes of *Kaempferia pandurata*. Asian Pacific Journal of Tropical Disease. 3: 401-404.

Tekeli A, Celik L, Kutlu H and Gorgulu M 2006. Effect of dietary supplemental plant extracts on performance, carcass characteristics, digestive system development, intestinal microflora and some blood parameters of broiler chicks. Proceedings of 12th European Poultry Conference:10-14.

Torki M, Sedgh-Gooya S and Mohammadi H 2018. Effects of adding essential oils of rosemary, dill and chicory extract to diets on performance, egg quality and some blood parameters of laying hens subjected to heat stress. Journal of Applied Animal Research. 46: 1118-1126.

Traber MG 2007. Vitamin E regulatory mechanisms. Annu. Rev. Nutr. 27: 347-362.

Tufarelli V, Casalino E, D'Alessandro AG and Laudadio V 2017. Dietary phenolic compounds: Biochemistry, metabolism and significance in animal and human health. Current Drug Metabolism. 18: 905-913.

USDA A 2000. United States standards, grades, and weight classes for shell eggs, ams 56.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J 2007. Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology. 39: 44-84.

Van Acker SA, Koymans LM and Bast A 1993. Molecular pharmacology of vitamin E: structural aspects of antioxidant activity. Free Radical Biology and Medicine. 15: 311-328.

Van Doan H, Hoseinifar SH, Chitmanat C, Jaturasitha S, Paolucci M, Ashouri G, Dawood MA and Esteban MÁ 2019. The effects of Thai ginseng, *Boesenbergia rotunda* powder on mucosal and serum immunity, disease resistance, and growth performance of Nile tilapia (Oreochromis niloticus) fingerlings. Aquaculture. 513: 734388.

Van den Brand H, Parmentier HK, and Kemp B 2004. Effects of housing system (outdoor vs. cages) and age of laying hens on egg characteristics. British Poultry Science. 4: 745–752.

Vandghanooni S, Forouharmehr A, Eskandani M, Barzegari A, Kafil V, Kashanian S and Ezzati Nazhad Dolatabadi J 2013. Cytotoxicity and DNA fragmentation properties of butylated hydroxyanisole. DNA and cell biology. 32: 98-103.

Wang X-c, Wang X-h, Wang J, Wang H, Zhang H-j, Wu S-g and Qi G-h 2018. Dietary tea polyphenol supplementation improved egg production performance, albumen quality, and magnum morphology of Hy-Line Brown hens during the late laying period. Journal of animal science. 96: 225-235.

Wang X, Wu S, Cui Y, Qi G, Wang J and Zhang H 2017. Effects of dietary tea polyphenols on performance, egg quality and antioxidant ability of laying hens. Chinese Journal of Animal Nutrition. 29: 193-201.

Wenk C 2003. Herbs and botanicals as feed additives in monogastric animals. Asian-Australasian Journal of Animal Sciences. 16: 282-289.

Yalcın S, Onbaşılar EE, Reisli Z and Yalcın S 2006. Effect of garlic powder on the performance, egg traits and blood parameters of laying hens. Journal of the Science of Food and Agriculture. 86: 1336-1339.

Yang L, Tan G-Y, Fu Y-Q, Feng J-H and Zhang M-H 2010. Effects of acute heat stress and subsequent stress removal on function of hepatic mitochondrial respiration, ROS production and lipid peroxidation in broiler chickens. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 151: 204-208.

Yara S, Lavoie J-C, Beaulieu J-F, Delvin E, Amre D, Marcil V, Seidman E and Levy E 2013. Iron-ascorbate-mediated lipid peroxidation causes epigenetic changes in the

antioxidant defense in intestinal epithelial cells: impact on inflammation. PloS one. 8: e63456.

Yasar S 1999. Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. British poultry science. 40: 65-76.

Yilmazer-Musa M, Griffith AM, Michels AJ, Schneider E and Frei B 2012. Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. Journal of agricultural and food chemistry. 60: 8924-8929.

Yitbarek MB 2015. Phytogenics as feed additives in poultry production: a review. International Journal of Extensive Research. 3: 49-60.

Yusuf NA, M Annuar MS and Khalid N 2013. Existence of bioactive flavonoids in rhizomes and plant cell cultures of *Boesenbergia rotund*'(L.) Mansf. Kulturpfl. Australian Journal of Crop Science. 7: 730-734.

Zhang J, Zhang C, Chen X and Quek SY 2020a. Effect of spray drying on phenolic compounds of cranberry juice and their stability during storage. Journal of Food Engineering. 269: 109744.

Zhang J, Zhang M, Liang W, Geng Z and Chen X 2020b. Green tea powder supplementation increased viscosity and decreased lysozyme activity of egg white during storage of eggs from Huainan partridge chicken. Italian Journal of Animal Science. 19: 586-592.

Zhao X, Yang Z, Yang W, Wang Y, Jiang S and Zhang G 2011. Effects of ginger root (Zingiber officinale) on laying performance and antioxidant status of laying hens and on dietary oxidation stability. Poultry science. 90: 1720-1727.

Zhong R and Zhou D 2013. Oxidative stress and role of natural plant derived antioxidants in animal reproduction. Journal of integrative agriculture. 12: 1826-1838.

Zhou B, Wu L-M, Yang L and Liu Z-L 2005. Evidence for α-tocopherol regeneration reaction of green tea polyphenols in SDS micelles. Free Radical Biology and Medicine. 38: 78-84.

Zhu Y, Wang J, Ding X, Bai S, Qi S, Zeng Q, Xuan Y, Su Z and Zhang K 2020. Effect of different tea polyphenol products on egg production performance, egg quality

and antioxidative status of laying hens. Animal Feed Science and Technology. 267: 114544.

VITA

NAME	Miftah Nurfarid
DATE OF BIRTH	30 March 1995
PLACE OF BIRTH	Subang
INSTITUTIONS ATTENDED	Chulalongkorn university
HOME ADDRESS	Street Wanasuka, Sanca, Ciater District, Subang Regency West Java Indonesia 41281